TEMPORAL AND SPATIAL DISTRIBUTION OF GRAZERS AND KELP PHLOROTANNINS IN KACHEMAK BAY, ALASKA

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A

THESIS

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Abstract

The potential influence of grazers on the density and distribution of kelp thalli is substantial and may be mediated by kelp phlorotannins serving in a defensive role. The purpose of this study was to determine how temporal and spatial phlorotannin patterns in four kelp species in Kachemak Bay, Alaska, are influenced by density and distribution of the gastropod grazer *Lacuna vincta* and environmental variables. Temporal phlorotannin patterns from June 2004 through December 2005 were mainly explained by the life history of particular kelp species as well as strong correlations with light attenuation and nitrate concentration. The Optimal Defense Theory of resource allocation to differentiated tissues was supported by observations of higher proportional allocation to attachment structures and meristematic tissue in all kelp species. *Lacuna vincta* distribution was not clearly related to phlorotannin content and therefore, grazer habitat and food choices may be influenced by the combination of high phlorotannin content, tissue toughness and/or nutritional content. Strong physical disturbances such as currents and wave action may supersede these factors and drive grazer distribution. An understanding of the biological and physical factors affecting phlorotannin content and distribution in kelp thalli may suggest reasons for temporal and spatial kelp bed variability.

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Preface

The success of this thesis is due in large part to the efforts of my graduate committee members, Drs. Brenda Konar and Rolf Gradinger, and my graduate advisor, Dr. Katrin Iken. Most especially, Katrin's tireless dedication, offerings of advice, and unselfish and caring demeanor have made my graduate experience with the School of Fisheries and Ocean Sciences highly enjoyable and memorable. For this, I am forever grateful. Field research at the Kasitsna Bay Lab was made possible by logistical support from Mike and Connie Geagel. The completion of nineteen successive months of field sampling was enabled by the reliable presence of Nick Harman, whose generous assistance contributed immensely to the achievement of this project. Diving support from Dr. Katrin Iken, Heloise Chenelot, Jon Snyder, Becca Bryan, Ben Daly and John Brewer is also very much appreciated. Statistical advice was provided by Drs. Ed Murphy, Dana Thomas and Dean Kildaw. Reviews from three anonymous reviewers greatly improved the contents of two submitted manuscripts.

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Chapter 1 - General introduction

1.1 Background

The existence of kelp in the sublittoral zone is vital to the maintenance of healthy nearshore ecosystems at temperate and subpolar latitudes. Kelp forests are extremely productive, releasing approximately 600-1300 g C m^{-2} into the coastal environment each year (Dayton 1985). They also contribute to cycling of dissolved and particulate nutrients by way of cell leakage during growth (Khailov and Burlakova 1969), sloughing of live tissue (Mann 1972) and degradation of senescent tissue from attached or drifting thalli (Newell et al. 1980). Kelps provide habitat and/or food for various epiphytes and grazers (Nicotri 1980; Pavia et al. 1999; Jørgensen and Christie 2003), and act as refuge and nursery grounds for juvenile fishes and invertebrates (Leighton 1971; Norton 1971; Schiel and Foster 1986). This diverse community of lower trophic level consumers provides food resources to higher trophic level carnivores (reviewed by Steneck et al. 2002).

The presence, abundance and distribution of kelp is strongly influenced by temporal and spatial heterogeneity of physical and biological factors (Dayton 1985). Kelp thalli tend to persist most readily in environments with high nutrient concentrations, ample light, low temperature and a moderate amount of water movement for transport of nutrients and algal spores (Dayton 1985). Seasonality of nutrients, light and temperature is extreme along the Alaskan coast, as is the density of herbivores. Grazers of the nearshore environment such as snails, limpets, chitons, sea urchins and amphipods are capable of consuming kelp tissues that are vital to survival and often difficult to replace (Schiel and Foster 1986 and references therein). Snails and sea urchins, specifically, may have profound negative effects on individual thalli and may even affect the presence and density of entire kelp beds (Fralick et al. 1974; Dean et al. 1984).

Over evolutionary time, many kelp species have developed defensive mechanisms such as increased tissue toughness or calcification and the production of distasteful chemical compounds that reduce feeding by grazers (Amsler and Fairhead 2006). The

effectiveness of these defense mechanisms in deterring herbivores has been mixed in studies using snails (Steneck and Watling 1982; Steinberg 1985; Steinberg and van Altena 1992) and amphipods (Hay et al. 1994; Poore 1994). Defensive chemicals may have varying effects on grazers based on concentration (Johnson and Mann 1986; Hay et al. 1994; Pavia and Toth 2000a), structure (Boettcher and Targett 1993) and herbivore susceptibility (Steinberg 1988; Targett and Arnold 1998).

The most common secondary metabolites associated with grazer deterrence in kelps are phlorotannins (Targett and Arnold 1998; Amsler and Fairhead 2006). Phlorotannins are polymers of phloroglucinol (1,3,5-trihydroxybenzene) (Ragan and Glombitza 1986; Arnold and Targett 2002) and possess primary metabolic roles in cell wall construction (Schoenwaelder and Clayton 1999) and wound healing (Lüder and Clayton 2004). Phlorotannins also are attributed to secondary metabolic roles in chemical defense that reduce assimilation efficiency in herbivore guts (Targett and Arnold 1998), but may also protect against damage by UV radiation (Pavia et al. 1997) and prevent or minimize settlement of larvae, epiphytes and bacteria (Ragan and Glombitza 1986; Wikström and Pavia 2004). Phlorotannins that are active in such secondary, protective functions are sequestered in physodes, vesicles used specifically for phlorotannin storage that prevent binding with other cellular components (Schoenwaelder 2002). These reactive phlorotannins can be extracted from algal tissue and quantified using spectrophotometric methods to evaluate their ecological roles.

Phlorotannin content in brown algae can vary considerably on multiple spatial and temporal scales (Ragan and Glombitza 1986; Van Alstyne et al. 1999b; Amsler and Fairhead 2006). Latitudinal differences in phlorotannin investment may exist between hemispheres (Steinberg 1989; Targett et al. 1992) or between temperate and tropical communities (Steinberg 1986; Van Alstyne and Paul 1990). Differences between geographic locations may be attributed to the temporal predictability of grazer presence, the effectiveness of the deterrent compound in minimizing herbivory (Boettcher and Targett 1993), and varying environmental conditions (Ragan and Glombitza 1986; Van Alstyne et al. 1999a). Within specific regions, characteristic physical factors such as

water circulation (Dayton 1985), temperature, salinity (Ragan and Glombitza 1986), irradiance (Pavia et al. 1997; Abdala-Díaz et al. 2006) and nutrient concentrations (Yates and Peckol 1993; Cronin and Hay 1996a; Pavia and Toth 2000b) can affect variability in phlorotannin concentrations. Depth has also been deemed influential in affecting phlorotannin content as currents, tidal surge and grazer distribution are typically not vertically uniform throughout the sublittoral environment (Ragan and Glombitza 1986; Fairhead et al. 2005). Temporal variations in these physical and biological factors may also have profound effects on phlorotannin content (Ragan and Glombitza 1986) and are most pronounced in the highly seasonal light environment of high latitude regions (see Chapter 2). In a small geographic area, inter-species phlorotannin variability may be influenced by kelp life history strategy (see Chapter 2). Intra-species variability can be affected by thallus age and size (Ragan and Glombitza 1986; Van Alstyne et al. 2001; Pavia et al. 2003). Within-thallus phlorotannin content may vary between tissue types (Tugwell and Branch 1989; Fairhead et al. 2005), in localized tissue regions (see Chapter 3) and even between cell layers (Tugwell and Branch 1989; Lüder and Clayton 2004). In summary, high phlorotannin variability on multiple scales often complicates spatial and temporal comparisons of intra- and interspecies patterns.

A topic of debate is the presumed costs associated with production, storage and metabolic turnover of phlorotannins. The underlying premise behind many ecological theories is that organisms have a finite amount of resources that they need to allocate to various life functions such as growth, maintenance, reproduction and defense (Cronin 2001). Since allocation of resources to one function will divert them from other functions, the relative partitioning of resources to various life processes may be closely associated to the benefits received by the individual based on this allocation strategy. However, an accurate assessment of the cost of resource allocation is problematic due to the difficulty in selecting the correct "currency" for measuring cost. A comprehensive determination of cost should involve factors like metabolite synthesis as well as turnover and exudation rates (Arnold and Targett 1998; Cronin 2001; Stamp 2003). The vast majority of phlorotannin research has addressed cost in terms of static measurements of phlorotannin

concentrations, though a few recent studies have noted the importance of microscopic examination of physodes and measurement of carbon uptake and assimilation into phlorotannins (Arnold and Targett 1998; Arnold and Targett 2003; Lüder and Clayton 2004).

Patterns of phlorotannin content and distribution within and between seasons are often related to a variety of ecological theories, most commonly the Carbon-Nutrient Balance Hypothesis (CNBH, Herms and Mattson 1992), Optimal Defense Theory (ODT, Rhoades 1979) and Induced Defense Theory (IDT, Harvell 1990). On a temporal scale, the CNBH presumes that carbon-based defenses are least costly to produce when nutrients such as nitrogen are in short supply and thus C:N is high (Bryant et al. 1983). On a spatial scale, the ODT predicts that those tissue types that are most important to individual fitness and are more difficult to replace will be the most highly defended (Rhoades 1979). In addition, risk of grazing attack on particular tissue types may also influence differential production of chemical defenses (Rhoades 1979). The IDT presumes that an individual thallus located in a region where grazing pressure is unpredictable or periodic will possess the ability to increase defensive chemical concentrations upon tissue wounding (Harvell 1990; Herms and Mattson 1992). Inducible defenses are generally assumed to be more cost-efficient than constitutive defenses, which are constantly produced regardless of the presence or absence of grazers (Clark and Harvell 1992; Herms and Mattson 1992).

1.2 Study justification

Large-scale comparisons of phlorotannin allocation within and between individuals and species have yielded ecologically important results. However, trends may be obscured by the considerable variability on these large scales in physical and biological factors affecting phlorotannin production and may be flawed by low resolution of spatial and temporal replication. Consequently, there is a need for small-scale studies employing multiple species within a localized region that are exposed to similar environmental conditions, community composition, and habitat type. Such work will

identify if phlorotannin distribution trends and the factors driving them are consistent on a regional spatial scale and across species. The purpose of this study was to investigate temporal and spatial phlorotannin content with respect to grazer density and distribution and physical parameters in several Alaskan subtidal kelp species. Most existing small-scale phlorotannin studies have utilized Fucalean brown algae and employed only a single species (e.g. Yates and Peckol 1993; e.g. Pavia and Åberg 1996; Borell et al. 2004). This study instead focuses on kelp species and also adds a high latitude perspective to phlorotannin research that has occurred mainly along low to mid-latitude temperate coastlines (e.g. Cronin and Hay 1996b; Van Alstyne et al. 1999b; Abdala-Díaz et al. 2006).

The dynamic nature of kelp beds in the study environment, the southern shore of Kachemak Bay, Alaska, has been demonstrated since the late 1970's by changes in species composition and density of the canopy-forming species *Nereocystis luetkeana* (Mertens) Postels et Ruprecht (Schoch and Chenelot 2004) and *Alaria fistulosa* Postels & Ruprecht (Dubois, personal observation based on report by Trasky et al. 1977). Though the reasons for these changes are largely unknown, herbivorous damage has been implicated in the reduction of algal biomass in other studies (Taylor 1998; Iken 1999) and may be the cause within the study environment. The predominant grazer in Kachemak Bay is the annual mesogastropod, *Lacuna vincta* (Montagu), which has been shown to destroy *N. luetkeana* canopy (Chenelot 2003) and decimate understory kelp blade tissue (Fralick et al. 1974; Johnson and Mann 1986). The gastropod *Calliostoma ligatum* (Gould) is also abundant in the study environment and is widely distributed throughout the kelp understory (Dubois, personal observation). This species may feed omnivorously (Perron 1975) and has a more constant presence throughout the year than does *L. vincta* (Dubois, personal observation). These two grazers, in addition to other less abundant snail, limpet, chiton, amphipod and sea urchin species, can be observed in Kachemak Bay on the canopy-forming kelp *N. luetkeana* and the understory kelps *Agarum clathratum* Dumortier, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (formerly *Laminaria saccharina* (Linnaeus) J.V. Lamouroux) and *Saccharina*

subsimplex (Setchell & N.L. Gardner) Widdowson, S.C. Lindstrom & P.W. Gabrielsen (formerly *Laminaria bongardiana* Postels & Ruprecht). These four conspicuous kelp species together comprise a large proportion of the macroalgal biomass and are common in the shallow subtidal environment of the study area.

1.3 Study objectives

The main purpose of the study was to assess temporal and spatial phlorotannin content in *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* thalli at two locations within Kachemak Bay in relation to *L. vincta* and *C. ligatum* density and distribution as well as environmental variables. The following objectives were used to test the hypotheses that 1) the studied kelp species produce reactive phlorotannins and that 2) between individuals during the summer, the concentration of these compounds is negatively correlated to *L. vincta* and *C. ligatum* density:

1) Determine the seasonal presence and content of phlorotannins in holdfast, meristematic stipe, meristematic blade, non-meristematic blade, degenerating blade and reproductive tissue in the four kelp species
2) Quantify seasonal *L. vincta* and *C. ligatum* density on the abovementioned tissue types of the four kelp species

Based on the assumption that kelp phlorotannins deter feeding by *L. vincta* and *C. ligatum*, I also hypothesized that 3) a negative relationship would exist between phlorotannin content and the amount of tissue consumed by these grazer species. I sought to support this hypothesis by comparing phlorotannin content in sections of meristematic stipe and blade tissue of each kelp species to relative palatability in no-choice feeding assays. According to the hypothesis, *L. vincta* and *C. ligatum* should feed proportionally less on kelp tissue with higher phlorotannin content and vice versa. I also anticipated that differences in physical factors between summer and winter would be reflected in phlorotannin content. As predicted by the CNBH and assuming that the production of

phlorotannins is energetically costly, I hypothesized that 4) phlorotannin content would be higher in the summer, when essential nutrients such as nitrogen are more limited, than in the winter. The following objectives were used to test these hypotheses:

3) Determine the relative palatability of meristematic stipe and blade segments of each kelp species based on mass of tissue consumed by *L. vincta* and *C. ligatum*
4) Measure temperature, salinity, light attenuation and bottom water nutrient concentrations within kelp beds at study locations and compare to summer and winter phlorotannin data

In order to assess within-thallus distribution of kelp phlorotannins in relation to the ODT, I hypothesized that 5) small-scale spatial allocation of phlorotannins in all kelp species is directly related to the importance of a particular tissue type to individual fitness. Holdfast and stipe tissues are necessary for substrate attachment and overwintering in some understory kelps, and meristematic blade and reproductive tissues are vital for thallus elongation and spore production, respectively. Hence, these tissue types should contain higher phlorotannin content than non-meristematic tissues. Furthermore, assuming that meristematic blade tissue is of high value within kelp thalli, protection of this tissue by use of inducible phlorotannins is presumably beneficial to thallus fitness. Therefore, I hypothesized that 6) simulated snail grazing would induce phlorotannin production in meristematic blade tissue. The following objectives facilitated testing of these hypotheses:

5) Relate phlorotannin content in all kelp tissues to the model proposed by the ODT
6) Evaluate phlorotannin inducibility in meristematic blade tissue of two kelp species based on single and repeated simulated mechanical wounding events

The first four hypotheses are addressed in Chapter 2 and the final two hypotheses in Chapter 3 based on data from the four studied kelp species from Hesketh Island (59°

30.3' N, 151° 31.8' W) and Jakolof Bay (59° 28.1' N, 151° 32.0' W; Fig. 1.1). The study sites are located on the southern shore of outer Kachemak Bay, which is adjacent to lower Cook Inlet in south-central Alaska. Hesketh Island and Jakolof Bay are similar in terms of substrate type, habitat complexity and environmental conditions, and demonstrate analogous macrofloral and faunal communities with an abundance of all study kelp and grazer species.

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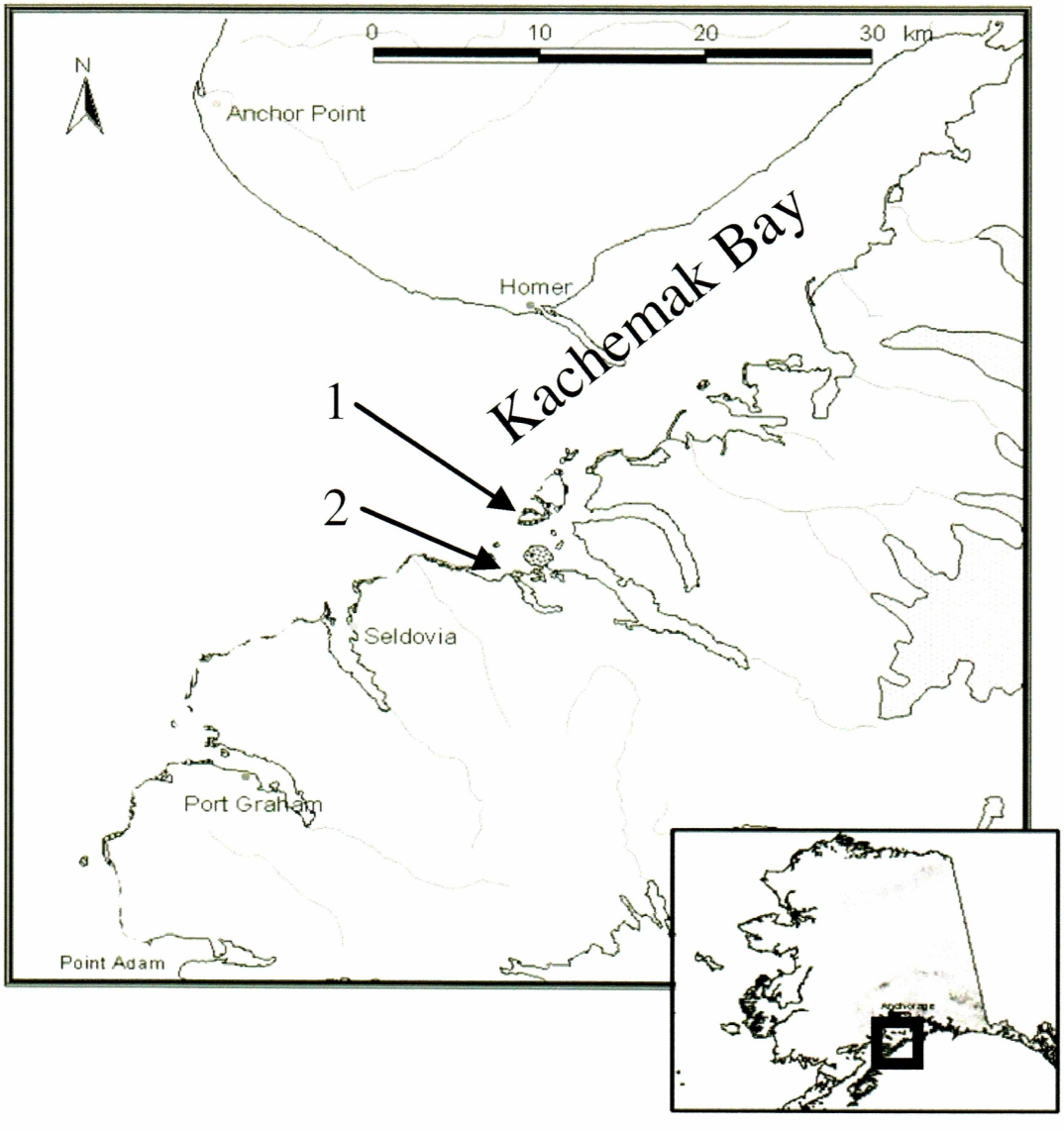

Fig. 1.1 Study locations at 1) Hesketh Island and 2) Jakolof Bay on the southern shore of Kachemak Bay, Alaska.

Chapter 2 - Seasonal variation in kelp phlorotannins in relation to grazer, light and nutrient dynamics in the Alaskan sublittoral zone[1]

2.1 Abstract

Kelps can produce secondary metabolites like phlorotannins, which may function to defend against herbivore attack; however, the factors regulating phlorotannin content within and between seasons in northeastern Pacific kelps are not well understood. This study assessed density of, and tissue consumption by the gastropods *Lacuna vincta* (Montagu) and *Calliostoma ligatum* (Gould) on the annual canopy-forming kelp *Nereocystis luetkeana* (Mertens) Postels & Ruprecht and the perennial understory species *Agarum clathratum* Dumortier, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders and *S. subsimplex* (Setchell & N.L. Gardner) Widdowson, S.C. Lindstrom & P.W. Gabrielsen in Kachemak Bay, Alaska (59° 29.0' N, 151° 31.9' W). We hypothesized that during summer kelp phlorotannin content is negatively correlated with *L. vincta* density and with tissue mass consumed based on presumed feeding deterrence by phlorotannins. *Lacuna vincta* was most dense on *N. luetkeana* thalli from June through September 2005 and consumed the greatest tissue mass of this low-phlorotannin species in palatability assays. However, correlations between *L. vincta* density in the field and phlorotannin content of each kelp species did not support the hypothesized relationship. *Calliostoma ligatum* was infrequently present on kelp thalli and did not feed in palatability assays. As proposed by the Carbon-Nutrient Balance Hypothesis, we also predicted that phlorotannins would be higher in summer than winter due to nitrogen limitation during summer. Differences in phlorotannin content between summer and winter existed in whole blade tissue within a species and demonstrated inverse trends between annual and perennial species. This may indicate that environmental factors such as light and nitrate as well as resource allocation strategies of

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kelp in relation to their life history strategies are more important in explaining seasonal phlorotannin patterns than is grazer abundance.

Key words: kelp phlorotannin content, *Lacuna vincta*, seasonal variability, Carbon-Nutrient Balance Hypothesis, palatability

2.2 Introduction

Mesograzers of the nearshore marine environment are capable of affecting the growth and reproductive abilities of individual macrophytes (Dean et al. 1984; Dethier et al. 2005) and even the composition and density of kelp forests (Fralick et al. 1974; Carney et al. 2005). Marine herbivores such as gastropods (Hay et al. 1989; Iken 1999; Granado and Caballero 2001) and crustaceans (Nicotri 1980; Duffy and Hay 1991; Pavia et al. 1999) may selectively aggregate and feed on macroalgal tissue based upon a complex balance of attractive and repellent factors. Mesograzer habitat and food choice is often attributed to algal morphological characteristics like shape, size and tissue toughness (Littler and Littler 1980; Steneck and Watling 1982; Hay et al. 1994), and to chemical properties related to nutritive value (Nicotri 1980; Cruz-Rivera and Hay 2003) and anti-herbivore defense (Paul et al. 2001).

Secondary metabolites that can serve as anti-herbivore defenses are widespread in temperate marine algae (for reviews see Hay 1996; Paul et al. 2001; Amsler and Fairhead 2006). Phlorotannins are polymers of phloroglucinol (1,3,5-trihydroxybenzene) and are ubiquitous in brown algae (Ragan and Glombitza 1986; Arnold and Targett 2002). The extent to which phlorotannins affect habitat and feeding selection by grazers seems to depend upon compound specificity and concentration as well as the susceptibility of the herbivore (Steinberg 1988; Targett and Arnold 1998). The effectiveness of phlorotannins as feeding deterrents is debated, with some studies demonstrating grazing reduction or inhibition (Geiselman and McConnell 1981; Pavia and Toth 2000b) and others showing negligible responses or even increased herbivory (Steinberg and van Altena 1992; Deal et al. 2003; Kubanek et al. 2004). Phlorotannins can also possess anti-biotic, anti-fungal,

anti-fouling and UV-protectant properties (Ragan and Glombitza 1986; Pavia et al. 1997; Wikström and Pavia 2004). In addition to these secondary functions, phlorotannins also are primary structural components of cell walls (Schoenwaelder and Clayton 1999) and act in wound healing (Lüder and Clayton 2004). Due to conflicting experimental results and an overall poor understanding of the potential interactions of primary and secondary functions, a true comprehension of phlorotannin roles in brown algae has yet to be achieved (Arnold and Targett 2002; Amsler and Fairhead 2006).

Brown algae show large species-specific variability in responses to factors that may regulate phlorotannin content (reviewed by Amsler and Fairhead 2006). Most studies concur that a variety of physical and biological variables act synergistically to affect distribution of phlorotannins within thalli (Hay 1996; Targett and Arnold 1998). Phlorotannins may be regulated by temporal variability in physical factors such as irradiance (Pavia and Toth 2000a), salinity (reviewed by Ragan and Glombitza 1986), nutrient regime (Yates and Peckol 1993; Cronin and Hay 1996), and water movement (Dayton 1985). Additionally, the presence of grazers may affect patterns of phlorotannin production if the macroalgal species in question demonstrates inducible defenses upon herbivorous damage (Hammerstrom et al. 1998; Pavia and Toth 2000b; Jormalainen et al. 2003; Hemmi et al. 2004).

Terrestrial ecological theories like the Carbon-Nutrient Balance Hypothesis (CNBH) address patterns of defensive chemical production based on resource availability and have been applied to marine systems with mixed success (for reviews see Cronin 2001; Amsler and Fairhead 2006). Inherent to the CNBH is the assumption that trade-offs exist between primary (growth) and secondary (defense) life functions and that growth requirements take precedence over defense (Coley et al. 1985; Tuomi et al. 1991; Herms and Mattson 1992). The CNBH predicts that photosynthetic rates will exceed growth rates in plants when nutrients such as nitrogen are limiting, and therefore a surplus of carbohydrates will lead to synthesis of carbon-based compounds such as tannins (Bryant et al. 1983; Hamilton et al. 2001; Arnold and Targett 2002). The application of the CNBH to phlorotannin content within and between species may help to explain seasonal

patterns of phlorotannin content in subtidal kelp communities (Coley et al. 1985; Peckol et al. 1996; Pavia et al. 2002).

In the present study, we investigated density and distribution on, and tissue consumption of the subtidal kelp species *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* (formerly *Laminaria saccharina*) and *S. subsimplex* (formerly *L. bongardiana*) by the gastropod grazers *Lacuna vincta* and *Calliostoma ligatum* based on presumed feeding deterrence by phlorotannins. We hypothesized that during the summer a negative relationship would exist between phlorotannin content in, and *L. vincta* and *C. ligatum* density on kelp tissue. Therefore, kelp thalli possessing high phlorotannin content would be associated with low densities of *L. vincta* and *C. ligatum*, while greater gastropod densities would be observed on kelp species with low phlorotannin content. This predicted negative relationship was also assessed using laboratory palatability assays with *L. vincta* and *C. ligatum* and kelp tissues of known phlorotannin content. Additionally, in accordance with the CNBH we hypothesized that phlorotannin content in kelp tissues of all four species would be higher during the summer than in the winter due to the limitation of nutrients essential to growth in summer in the study environment. Water column nutrients and light were measured to support our prediction.

2.3 Materials and methods

2.3.1 Study species and sites

The herbivorous gastropod grazer *Lacuna vincta* (Montagu) occurs abundantly throughout the northeastern Pacific from the shallow subtidal to lower intertidal zones (Duggins et al. 2001; Chenelot 2003; Carney et al. 2005) and is typically less than 6 mm in shell length in the study area. *Lacuna vincta* grazing has been reported to significantly damage canopy (Duggins et al. 2001; Chenelot 2003; Carney et al. 2005) and understory kelp thalli (Fralick et al. 1974; Johnson and Mann 1986). The omnivorous gastropod *Calliostoma ligatum* (Gould) may be up to four times larger than *L. vincta* and is generally less numerous but widespread in the shallow subtidal environment. *Calliostoma*

ligatum occupies much of the northeastern Pacific (Hammerstrom et al. 1998) but is rarely observed in the canopy (Perron 1975; Duggins et al. 2001; Carney et al. 2005). In addition to algal tissue, this species may feed on detritus and epiphytic organisms such as diatoms and hydroids (Perron 1975). Both of these grazer species are abundant in the study environment of Kachemak Bay, Alaska, and commonly reside on the predominantly annual kelp *Nereocystis luetkeana* (Mertens) Postels & Ruprecht and the perennial understory kelps *Agarum clathratum* Dumortier, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders and *S. subsimplex* (Setchell & N.L. Gardner) Widdowson, S.C. Lindstrom & P.W. Gabrielsen. The perennial kelp species may persist through the winter as complete individuals or as stipes (O'Clair and Lindstrom 2000; Chenelot 2003). All four kelp species occur in abundance within the study area (Appendix 2.A) and comprise the majority of the macroalgal community.

Field studies occurred off the western end of Hesketh Island (59° 30.3' N, 151° 31.8' W) and at the mouth of Jakolof Bay (59° 28.1' N, 151° 32.0' W) within Kachemak Bay on the south-central coast of Alaska. Study sites were located approximately 5 m below mean low water neap tide levels (MLWN) and were exposed to inclement weather from the open bay. Study locations were similar physically (temperature, salinity, light exposure, nutrient regime) and biologically (algal and grazer assemblages), though the Hesketh Island site was more exposed to wave surge while Jakolof Bay was more often subjected to tidal currents given its proximity to an adjacent channel.

2.3.2 Gastropod and kelp surveys

Collection of *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* thalli for phlorotannin analysis occurred at Hesketh Island every two weeks in summer (June to September of 2004 and 2005) and monthly in winter (December 2004 to March 2005). *Lacuna vincta* and *C. ligatum* densities were measured on the same sampling dates as above but during 2005 only. Due to logistical constraints, sampling at Jakolof Bay took place half as frequently as at Hesketh Island. Gastropod density was determined by enumeration of *L. vincta* and *C. ligatum* on the entire surface of the stipe and within three

100 cm^2 quadrats per blade of 3 randomly selected individuals per kelp species along one 30 m transect. For counts on *N. luetkeana*, quadrats were placed along the length of one blade which seemed representative of average gastropod density. Due to the patchy distribution of grazers on kelp, some sampling error in estimation of gastropod density was likely introduced as a result of this sampling protocol. Based on the constant presence but low density of *C. ligatum* on individual kelp thalli and the difficulty of distinguishing juveniles from *Margarites pupillus* (Gould) in the field, *C. ligatum* density data are not presented.

The same kelp individuals observed for gastropod densities were removed from the substrate below the holdfast and kept in cold seawater in the dark for transport ($n = 3$ thalli per species per study site). In the lab, each thallus was promptly measured to determine stipe length and circumference as an estimate of surface area available for grazing. Blade lengths and widths of all species as well as pneumatocyst circumference for *N. luetkeana* were also measured. Tissue segments (<1.5 g each) from meristematic stipe (taken from just above the holdfast), meristematic blade, non-meristematic blade and degenerating blade were excised from each thallus for phlorotannin analysis. Determinations of blade tissue type were made by visually dividing the blade from the base to tip into thirds and then sampling just to the side of the midrib or central region in the middle of each segment. Blade tissue data from the three regions were pooled to represent whole blade phlorotannin content per species. Excised tissue was weighed and kept frozen at -20 °C until phlorotannin analysis.

2.3.3 Purification of phlorotannin standards

Preparation of phlorotannin standards and analysis of kelp tissue phlorotannin content followed the 2, 4-dimethoxybenzaldehyde (DMBA) method described by Stern et al. (1996). This procedure was selected instead of a frequently used Folin-type assay to minimize spectrophotometric "interference" from reactions between non-phlorotannin compounds present in the extract and the color reagent. In addition, the DMBA method uses species-specific phlorotannins as standards that are purified from the various kelps

from the study locations to account for reactivity variation of different phlorotannin structures.

Frozen kelp tissue was ground using a Power Gen 500 homogenizer. Homogenized tissue was extracted on ice and in the dark in 80 % HPLC-grade methanol while rocked. Solvent was replaced 3 - 4 times within approximately 12 h. The combined extracts were filtered through 8 μm cellulose filters (Whatman) and solvents evaporated (Labconco rotary evaporator) under reduced pressure at 35 °C. The dried sample was triturated with 100 % methanol and filtered as above to remove salts. The solution was then partially dried and the remaining mixture adsorbed onto microcrystalline cellulose and completely dried. The extract-laden cellulose was placed onto a bed of clean microcrystalline cellulose and rinsed with toluene until the filtrate appeared clear. A solution of 2:1 acetone-water removed the phlorotannins from the cellulose and this extract was then dried. Each standard was freeze dried for 48 h and then ground to a fine powder.

Two phlorotannin standards were made for each species as mentioned above using tissue collected from both study sites. Replicate standard curves for each species were determined using a 0-500 μg μl^{-1} range of phlorotannin mass to methanol volume. The use of a mean slope from multiple replicates accounted for some procedural variability in standard curves, though differences among replicates were low (Table 2.1).

2.3.4 Measurement of kelp phlorotannins

Kelp tissue segments of known wet weight were homogenized individually and extracted overnight in 80 % methanol at -20 °C (Stern et al. 1996). The DMBA assay working reagent was freshly made before each set of assays by combining equal volumes of 2, 4-dimethylbenzaldehyde (2 g x 100 ml^{-1} solution) and hydrochloric acid (16 ml x 100 ml^{-1} solution) in glacial acetic acid. Between 200 and 400 μl of extract (depending upon kelp species) were then added to 2.5 ml of working reagent and 10 μl of *N, N*-dimethylformamide. Solutions were incubated at 30 °C for exactly 1 h and then absorbance at 510 nm was determined spectrophotometrically (Thermospectronic

Genesys 5). Because some color change may occur in the absence of phlorotannins, samples were measured against blanks of the same volume in all assays. Calculation of phlorotannin content as a percentage of dry mass (% DM) was based on the ratio of wet:dry mass for each tissue type per kelp species (n = 6) after drying at 60 °C for 48 h.

2.3.5 Feeding assays

Laboratory feeding assays with *L. vincta* and *C. ligatum* were conducted during the summer of 2004 and 2005 to test for the relative palatability of meristematic stipe or meristematic blade tissue of the four kelp species in no-choice experiments. Grazers were taken from areas adjacent to the study sites and allowed to clear guts for 24 h in running seawater tanks. Prior to the start of each experiment, 10 individuals per kelp species were collected from Hesketh Island or Jakolof Bay and 3 adjacent 1.5 ± 0.1 g pieces of stipe and blade tissue excised from each individual. One piece of each tissue type was used in a treatment container, the second in a control container and the third frozen for corresponding phlorotannin analysis. Fifty *L. vincta* or 3 *C. ligatum* of average size were placed in a treatment container (n = 10) with a piece of either stipe or blade tissue of a kelp species. The quantity of *L. vincta* in each container was based on the maximum density observed in the field on a tissue area similar to that used in feeding assays. A paired container per kelp species and tissue type held only a tissue segment as a control to account for autogenic weight changes in kelp tissue (Peterson and Renaud 1989). Containers were floated in flow-through seawater tanks to maintain ambient temperature and natural light regimes, and water within containers was replaced every 24 h. Relative mass change after 72 h was determined by the difference in wet mass of tissue exposed to grazers minus the mass change of ungrazed control segments.

Results with *C. ligatum* demonstrated kelp mass increases in treatment containers with *N. luetkeana* and *A. clathratum* tissue relative to controls, so additional trials were completed with this grazer and these two kelp species. To test for possible non-grazing effects (e.g., respiration, defecation) of gastropods on the mass change of kelp tissue, treatment containers (n = 10) were divided with mesh to separate 3 *C. ligatum* and the

stipe or blade segment. This design allowed for water exchange between the compartments without any direct grazing on kelp tissue. Control containers (n = 10) were identical in construction but did not include grazers. Analyses of variance (ANOVA) showed that wet mass change in stipe segments of *N. luetkeana* in the presence and absence of grazers was not significantly different ($p > 0.05$). Blade tissue was marginally different ($p = 0.04$), though this result was attributed to small sample size due to accidental loss of replicates. *Agarum clathratum* tissue was not significantly impacted by non-grazing effects ($p > 0.05$ for stipes and blades). Therefore, correction factors for palatability mass changes due to non-grazing effects were not incorporated.

2.3.6 Environmental variables

Nutrient concentrations, light attenuation, temperature and salinity were measured at each field sampling. Water samples obtained ~1 m above the substrate were used for salinity measurements as well as analysis of nitrate, ammonium, phosphate and silicate concentrations (Alpkem model RFA-300 continuous nutrient analyzer) based on colorimetric techniques (Whitledge et al. 1981). Light profiles were taken with a LiCor 193SA spherical sensor to determine irradiance ($\mu E\ m^{-2}\ s^{-1}$) just above and below the water surface and throughout the water column in 1 m increments. Percent attenuation of surface light was standardized for 5 m depth for all sampling occasions to approximate attenuation in the understory canopy. Since *N. luetkeana* blades are close to or at the water surface for a large proportion of their life cycle, percent attenuation at 1 m was used for this species. Maximum and minimum mean temperatures from a data logger deployed at Hesketh Island were used to establish "summer" (10.3 ± 0.10 °C) and "winter" (4.88 ± 0.07 °C) seasons, which will be henceforth defined as the months of June through September and December through March, respectively.

2.3.7 Statistical analyses

Prior to statistical assessments, parametric assumptions for ANOVAs were evaluated using residual plots and Shapiro-Wilk tests (Zar 1999). *Lacuna vincta* density was transformed by means of ranks on normal scores (Conover 1999). Density data were assessed separately for each site (Hesketh Island and Jakolof Bay) and tissue type (stipe and whole blade) using single-factor ANOVAs to determine differences in *L. vincta* density between kelp species during summer. Two-factor ANOVAs were used to assess seasonal differences in *L. vincta* density at each site, with season and kelp species as fixed orthogonal factors. Season was defined as summer or winter as noted above. Phlorotannin data were arcsine-square root transformed and evaluated separately for each site and tissue type as above for *L. vincta* density. Single-factor ANOVAs tested for phlorotannin differences between kelp species at each site during summer. Two-factor ANOVAs were used to determine phlorotannin content differences between summer and winter in kelp species at each site. Untransformed tissue mass and phlorotannin data from palatability assays were analyzed separately for each grazer species (*L. vincta* or *C. ligatum*) and tissue type (meristematic stipe or meristematic blade) by single-factor ANOVAs with kelp species as the fixed factor. All post-hoc assessments of significant effects and interactions were made using the Tukey-Kramer method.

Pearson's product-moment correlations were used to separately assess the relationship between phlorotannin content of each kelp species and *L. vincta* density, environmental variables or tissue mass consumed in palatability assays. Correlations between phlorotannin content and *L. vincta* density at each site included only whole blade tissue data from the summer of 2005. Observations on stipe tissue and from winter were not regarded because of the infrequent occurrence of *L. vincta* on that tissue type and during that season. Environmental data included raw light attenuation and log-transformed nitrate and ammonium. Since nitrate availability is central to the predictions of the CNBH, log-transformed phosphate and silicate were dropped from the model before correlation analyses due to strong autocorrelation ($p < 0.001$). Due to low sample sizes at Hesketh Island and Jakolof Bay and a lack of significant differences in light,

nitrate and ammonium between sites, correlations between phlorotannin content of each kelp species and environmental variables were grouped for both sites. All statistical tests were conducted using SAS, v9.1 at $\alpha = 0.05$.

2.4 Results

2.4.1 *Lacuna vincta* density and distribution

Within the summer season at both sites, *L. vincta* demonstrated significantly higher density on *N. luetkeana* stipes as opposed to those of the understory species ($p < 0.01$ for each site; Fig. 2.1). *Lacuna vincta* density was higher on *N. luetkeana* whole blade tissue than any of the other kelp species at Hesketh Island, but this difference was only significant as compared to density on *A. clathratum* ($p < 0.05$; Fig. 2.1a). No significant density differences on whole blade tissue between kelp species were apparent at Jakolof Bay (Fig. 2.1b).

When present on kelp thalli, *L. vincta* were similarly or more dense on stipe tissue of all species during the summer than the winter at Hesketh Island (Fig. 2.1a) and Jakolof Bay (Fig. 2.1b). Significantly higher *L. vincta* density on stipe tissue during summer occurred only on *N. luetkeana* at Hesketh Island ($p = 0.04$; Fig. 2.1a). At Jakolof Bay, *L. vincta* were absent from *A. clathratum* and *S. latissima* stipes during summer and were less dense on *N. luetkeana* stipes than at Hesketh Island (Fig. 2.1b). Significant effects of kelp species and the season * kelp species interaction at Jakolof Bay were driven by high *L. vincta* density on *N. luetkeana* stipes relative to other species during summer (Table 2.2). In terms of whole blade tissue, there was an effect of season on *L. vincta* density at each site ($p < 0.01$; Table 2.2) and snails were significantly more dense during summer than winter on *N. luetkeana* at Hesketh Island ($p < 0.001$; Fig. 2.1a) and *S. subsimplex* at Jakolof Bay ($p < 0.01$; Fig. 2.1b). While summer density of *L. vincta* on *N. luetkeana* blades was lower at Jakolof Bay than Hesketh Island, snails at the former site were distributed proportionally more on blades of the perennial understory species (Fig. 2.1).

Greater temporal resolution of *L. vincta* density on all kelp species at both sites from March through December 2005 is presented in Appendix 2.B.

2.4.2 Phlorotannin content

Mean summer phlorotannin content in meristematic stipe tissue was significantly lower in *N. luetkeana* ($p < 0.05$; but not different from *S. subsimplex*) and higher in *S. latissima* thalli than in all other kelp species at both sites ($p < 0.05$ for each site; Fig. 2.2). Whole blade phlorotannin content was also lower in *N. luetkeana* thalli than in all other kelp species at both sites ($p < 0.05$ for each site), while all other species were similar within a site (Fig. 2.2).

No significant seasonal differences in meristematic stipe phlorotannin content existed in any kelp species at either study site (Fig. 2.2; Table 2.3). In general, *N. luetkeana* stipes contained insignificantly higher phlorotannin content in summer, but the understory species had similar phlorotannin contents in both seasons (Fig. 2.2). Significant effects of kelp species at each site were influenced by high *S. latissima* stipe phlorotannin content (Fig. 2.2; Table 2.3). In terms of whole blade tissue, *N. luetkeana* had higher phlorotannin content during summer at both sites, though this seasonal difference was only significant at Hesketh Island ($p < 0.001$; Fig. 2.2a). In contrast, *A. clathratum* and *S. latissima* whole blades contained higher phlorotannin content in winter at both sites, though this difference was only significant in *S. latissima* at Hesketh Island ($p = 0.04$; Fig. 2.2). *Saccharina subsimplex* phlorotannin content in whole blades was significantly higher during winter at Hesketh Island ($p < 0.01$; Fig. 2.2a) but demonstrated a non-significant reverse seasonal trend at Jakolof Bay (Fig. 2.2b). Greater temporal resolution of phlorotannin content in all kelp species at both sites from June 2004 through December 2005 is shown in Appendix 2.C.

2.4.3. Feeding assays

Grazing by *C. ligatum* was negligible in experimental trials despite continuous defecation, as evidenced by minimal wet mass changes in meristematic stipe and blade

tissues (Appendix 2.D). There was no significant effect of kelp species in either tissue type in *C. ligatum* assays. As much as 45% of the total wet mass of kelp tissue was consumed by *L. vincta* within 72 h and in general, a greater mass of meristematic blade tissue was consumed than meristematic stipe tissue in all kelp species (Fig. 2.3). When feeding on stipes of each kelp species, *L. vincta* was observed feeding on the cut ends and within holes created by grazing during the experiment. Consumption was significantly different between kelp species in both stipe and blade trials ($p < 0.01$ for each tissue type). *Lacuna vincta* consumed significantly more stipe mass of *N. luetkeana* than of *S. latissima* and *S. subsimplex* ($p < 0.05$; Fig. 2.3a), and more blade tissue of *N. luetkeana* than of any understory species ($p < 0.05$; Fig. 2.3b).

Phlorotannin content of tissues used in feeding experiments varied significantly between kelp species in both *C. ligatum* and *L. vincta* assays ($p < 0.01$ for each tissue type), with *N. luetkeana* and *S. subsimplex* meristematic stipes having the lowest and *S. latissima* stipes the highest phlorotannin content ($p < 0.05$; Fig. 2.3a, Appendix 2.D). Meristematic blade phlorotannins were low (<2.0 % DM) in all kelp species except for *A. clathratum* in *C. ligatum* assays (Appendix 2.D). Based on differences in tissue mass consumed by *L. vincta* and phlorotannin content in the four kelp species, the greatest consumption of stipe tissue occurred in tissue sections containing the lowest phlorotannin content (*N. luetkeana*) and the least amount of stipe tissue eaten held the highest phlorotannin content (*S. latissima*; Fig. 2.3a). A significant negative relationship between mass consumed by *L. vincta* and phlorotannin content occurred only with *S. latissima* stipes ($R^2 = 0.79$, $p < 0.01$). Linear regressions with meristematic blade tissue did not demonstrate a clear relationship between *L. vincta* grazing and tissue phlorotannin content in any of the kelp species. Based on the negligible feeding of *C. ligatum* in all trials, there is no correlative relationship between the amount of tissue consumed and phlorotannin content.

2.4.4. Environmental variables

Light attenuation (% of surface irradiance) at 5 m depth for the understory species was greatest in the summer and decreased by ~22 % in winter (Table 2.4). For *N. luetkeana* blades, attenuation at 1 m was comparable between summer and winter at ~39 %. Considerable variability and low sample size during winter likely contributed to the lack of a significant seasonal difference with respect to light attenuation. Nitrate, phosphate and silicate concentrations (μM) were significantly higher in winter (each $p < 0.001$). In contrast, ammonium values were insignificantly lower during winter (Table 2.4). Temperature ranged from approximately 4.5 to 4.8 °C in winter and 9.1 to 10.6 °C in summer and was significantly different between seasons at Hesketh Island ($p < 0.001$; Appendix 2.E) and Jakolof Bay ($p = 0.034$; data not shown). Salinity values were independent of season and not significantly different between summer and winter (Appendix 2.E) or between sites ($p = 0.554$).

2.4.5 Phlorotannin content in relation to *Lacuna vincta* density and environmental variables

Linear relationships between summer phlorotannin content and *L. vincta* density were not significant for any kelp species at either site, though a nearly significant correlation existed with respect to *N. luetkeana* at Hesketh Island ($R^2 = 0.21, p = 0.07$). The lack of a statistical relationship between phlorotannin content and *L. vincta* density may have been exacerbated by the high frequency of low grazer counts at the beginning and end of the summer season regardless of kelp tissue phlorotannin content.

An assessment of environmental factors revealed that phlorotannin content of *N. luetkeana* was significantly negatively correlated with nitrate concentration ($R^2 = 0.66, p < 0.001$). A significant positive relationship occurred between *N. luetkeana* phlorotannin content and light attenuation at 5 m ($R^2 = 0.20, p = 0.05$), but not at 1 m. Light attenuation at 5 m was negatively correlated with phlorotannin content in *A. clathratum* ($R^2 = 0.35, p = 0.01$) and *S. latissima* ($R^2 = 0.25, p = 0.02$), though not significantly with *S. subsimplex*. Temperature and salinity did not correlate with phlorotannin content in any kelp species.

2.5 Discussion

The presence and abundance of *L. vincta* was highly seasonal within Kachemak Bay. The sudden appearance of this species in summer demonstrated that high densities can be achieved within a matter of weeks. The greatest density observed at Hesketh Island (70.7 *L. vincta* 100 cm^{-2}) exceeded that documented in other studies of this grazer (Fretter and Manly 1977; Thomas and Page 1983; Chenelot 2003). *Lacuna vincta* was distributed predominantly on *N. luetkeana* blades as opposed to the understory species at Hesketh Island. This preference for residing in the canopy and on *N. luetkeana* was also noted with *L. vincta* in the San Juan Archipelago (Duggins et al. 2001; Carney et al. 2005). The lower density of *L. vincta* on *N. luetkeana* blades at Jakolof Bay may be due to the general migration of this grazer from the canopy to understory species in August of 2005. *Lacuna vincta* has been reported elsewhere to move to the understory one to three weeks post-settlement, thereby increasing their presence on bed-forming kelp in late summer (Martel and Chia 1991). However, in Jakolof Bay such downward migrations may have been accelerated by strong physical disturbances (storms) concurrent with large tides at the end of July 2005. Furthermore, strong currents at this site may have contributed to the lower density of *L. vincta* on *N. luetkeana* thalli as compared to Hesketh Island by dislodging grazers from exposed stipe and blade tissue. Duggins et al. (2001) showed that moderate tidal currents were capable of rapidly removing large proportions of *L. vincta* from *N. luetkeana* stipes. *Lacuna vincta* has been shown to utilize understory blades for shelter and protection from physical stresses such as water currents (Fretter and Manly 1977), which may support their almost exclusive appearance on the understory species at Jakolof Bay late in the summer of 2005.

The distribution of *L. vincta* in the algal community may indicate factors important to habitat and food selection for this species. Apart from protection from currents, habitat selection may also be driven by predation pressure (Hay et al. 1989; Duffy and Hay 1991). As a great majority of fish in the study environment are located within understory kelp (Hamilton and Konar 2006), preferential residence in the *N.*

luetkeana canopy may provide refuge from this high predation pressure close to the substrate. At Jakolof Bay, a trade-off in habitat features between the predation-protected canopy and the more physically protected understory may have driven *L. vincta* distribution. Also, nutritive value of tissue may (Steinberg 1985) or may not (Wakefield and Murray 1998; Granado and Caballero 2001) influence gastropod distribution. The most commonly used indicator of nutritive content in marine algae is the carbon to nitrogen ratio (C:N), with low values being indicative of higher nutritive content (Duffy and Hay 1991). Blade tissue C:N ratios from the Kachemak Bay study sites approximate 10.6 ± 0.43 for *N. luetkeana*, 11.7 ± 0.28 for *A. clathratum*, 17.6 ± 1.39 for *S. latissima* and 19.0 ± 1.82 for *S. subsimplex* (Appendix 2.F). Prior studies support the range of these C:N values for *N. luetkeana* (Atkinson and Smith 1983; Rosell and Srivastava 1985) and the related species *Agarum fimbriatum* (Duggins and Eckman 1997), though literature values for *S. latissima* and *S. subsimplex* are somewhat lower than reported in this study (Duggins and Eckman 1997; Gevaert et al. 2001). If C:N ratios are used as a proxy for nutritional content and a low ratio is more nutritious, *N. luetkeana* and *A. clathratum* are potentially the most and *S. latissima* and *S. subsimplex* the least attractive food of the four kelp species. Palatability trends from this study support these nutritional comparisons as *L. vincta* consumed more *N. luetkeana* and *A. clathratum* stipe and blade tissue relative to *S. latissima* and *S. subsimplex*.

The presence of phlorotannins may be a critical factor implicated in food selection by *L. vincta*. Northeastern Pacific kelps from this study exhibited detectable phlorotannin concentrations in stipe and whole blade tissue throughout the duration of field surveys. Mean annual phlorotannin values of all kelp species approximate those measured previously in northern Pacific or Atlantic brown algae (Ragan and Glombitza 1986; Targett et al. 1992; Van Alstyne et al. 1999), though the remarkably high content in *S. latissima* stipes is unprecedented. The ecological and physiological significance of these high phlorotannin values is unexplained as of yet. However, it is unlikely that methodological problems with stipe phlorotannin assays caused inflated values since the same laboratory procedures were used to produce phlorotannin values for all other kelp

species and tissue types that are within range of those reported in the scientific literature. The large variability in phlorotannin content in the studied kelps is also similar to other observations of substantial differences within (e.g. Pavia et al. 2003; Fairhead et al. 2005) and between (e.g. Ragan and Glombitza 1986; Van Alstyne et al. 1999) species.

As hypothesized, *L. vincta* density during summer at Hesketh Island was on average higher on stipes and blades of low-phlorotannin thalli (*N. luetkeana*) and lower on thallus tissues of those species containing higher phlorotannins (especially *A. clathratum*). In palatability assays, *L. vincta* consumed the most meristematic stipe and blade tissue of *N. luetkeana*, which had among the lowest phlorotannin levels (0.14-0.77 % DM) of the studied kelp species. Overall, blades were consumed more than stipes irrespective of kelp species as has been observed in previous studies of *L. vincta* (Johnson and Mann 1986; Toth and Pavia 2002). Phlorotannin content in stipes is often higher than in blades of kelp thalli (e.g. Ragan and Glombitza 1986; Tugwell and Branch 1989), which may explain the lower consumption of stipe tissue by *L. vincta*. Furthermore, since phlorotannins are located primarily in meristodermal tissue (Tugwell and Branch 1989; Lüder and Clayton 2004), feeding of *L. vincta* on medullary stipe tissue may indicate avoidance of phlorotannin-richer cortical regions (see also Johnson and Mann 1986). However, tissue mass consumed by *L. vincta* in feeding assays was not directly proportional to phlorotannin content of each kelp species; therefore, factors in addition to phlorotannins, such as tissue toughness or nutritive value may have affected palatability. Moreover, the hypothesized negative correlation between *L. vincta* density and kelp blade phlorotannins during summer was only apparent at Hesketh Island. Based on the lack of a consistent relationship between *L. vincta* and phlorotannin content in the four kelp species during summer in both field and laboratory observations, it is plausible that this grazer is not significantly deterred by the mere presence of phlorotannins in kelp tissue. Phlorotannins may have concentration-dependent effects on gastropods (Johnson and Mann 1986; Pavia and Toth 2000b), with mean phenolic levels less than 2 % DM not significantly deterring feeding (Hay et al. 1994). When offered phenolic-rich and poor algal species, the gastropod *Tegula funebralis* preferentially grazed on individuals with

phlorotannin content less than 1.65 % DM (Steinberg 1985). *Lacuna vincta* avoided *Laminaria* (now *Saccharina*) *longicruris* meristematic blade tissue (approximately 5.5 % DM) but grazed on all other tissue types with phlorotannin levels less than 1.0 % DM (Johnson and Mann 1986). Unfortunately, experiments using isolated phlorotannins to specifically test their effects on grazing could not be performed in the present study because *L. vincta* did not respond to any artificial food. Therefore, a true causal relationship between phlorotannins as quantitative chemical deterrents and *L. vincta* food consumption cannot be conclusively established. It is also possible that the observed relationship alternatively suggests that kelp did not respond appreciably to the presence of *L. vincta* by increasing blade phlorotannin content.

The lack of grazing by *C. ligatum* in palatability assays may be indicative of its more omnivorous feeding on diatoms, epiphytes and detrital film from the surface of kelp tissue. Support for this potential mixed diet as opposed to solely kelp tissue is evidenced by an enriched $\delta^{15}N$ stable isotope value of *C. ligatum* relative to *L. vincta* (Appendix 2.G). Three other species in the same genus have been classified as omnivores based on gut contents (Perron 1975) or elevated stable isotope values relative to strictly herbivorous gastropods (Fredriksen 2003). Additionally, the morphology of *C. ligatum*'s rhipidoglossan radula is not conducive to scraping leathery macrophytes due to its poor ability to apply force and excavate tough tissue (Steneck and Watling 1982). The potential for omnivorous behavior by *C. ligatum* in Kachemak Bay may be supported by the presence of this grazer on bryozoans and hydroids on understory kelp late in the summer (Dubois, personal observation).

The analysis of environmental factors demonstrated that *N. luetkeana* phlorotannins were significantly negatively correlated with water column nitrate and positively with light attenuation at 5 m. The lack of a correlation with light at 1 m was likely due to large variability in irradiance measurements near the water surface. The understory species, with the exception of *S. subsimplex*, were significantly negatively correlated with light attenuation at 5 m, which indicates an inverse relationship between

light and phlorotannin content in the perennial species as compared to the annual, *N. luetkeana*.

The CNBH predicts that environmental conditions under which growth is limited by low nitrogen but light is ample for photosynthesis will result in excess production of photosynthate, which can be allocated to secondary metabolite production (e.g. Bryant et al. 1983; Hamilton et al. 2001; Arnold and Targett 2002). The presence of defensive chemicals during periods of compromised growth may also serve to protect vulnerable tissue that would be difficult to replace if damaged by grazers (Herms and Mattson 1992). During the short summer in the North Pacific, nitrate is less available and light is more constant and intense, so species following the predictions of the CNBH should have higher phlorotannin content in the summer. By having the highest phlorotannin content during the season with the lowest nitrate concentrations, only *N. luetkeana* followed the model proposed by the CNBH. As an annual species, *N. luetkeana* has limited time to complete its life cycle and loss of tissue to grazing is potentially devastating. Hence, seasonal allocation of carbon sources to phlorotannin production may be vital for *N. luetkeana*. As discussed above, the effectiveness of phlorotannins against *L. vincta* may be weak but phlorotannins may still assist with *N. luetkeana* fitness against other grazers or in other ways.

The CNBH not only predicts an inverse relationship between nitrogen availability and secondary metabolite production but also that resources, when available, are first allocated to growth as opposed to defense (Coley et al. 1985; Tuomi et al. 1991; Herms and Mattson 1992). Growth as a costly process potentially competing for resources with phlorotannin production was not measured in this study. Nevertheless, the season of highest phlorotannin content in *N. luetkeana* blade tissue in Kachemak Bay likely coincided with the season of most rapid growth, as determined in Puget Sound for this species (Maxell and Miller 1996). Therefore, while *N. luetkeana* followed CNBH predictions based on nitrate-phlorotannin relationships, it contradicted the theory by simultaneously allocating resources into maximum growth and phlorotannin production. It can be argued, however, that the species exhibits a relative (if not absolute) trade-off in

resource allocation by producing overall low phlorotannin levels as compared to the understory species.

In contrast to the annual *N. luetkeana*, phlorotannins in the perennial species were negatively correlated with light attenuation and not significantly related to nitrate availability, therefore not following CNBH predictions. High seasonal variability in phlorotannin content in the understory species may have contributed to the lack of correlation with environmental factors such as nutrient concentrations. Nevertheless, the highest growth rates in perennial kelps such as *Agarum cribrosum* (now *A. clathratum*), *Laminaria* (now *Saccharina*) *longicruris* and *L.* (now *S.*) *digitata* typically occur during winter months (Mann 1972). Assuming similar growth patterns for *A. clathratum*, *S. latissima* and *S. subsimplex* thalli if they maintain blade tissue through the winter, these species demonstrated an approximately simultaneous allocation of resources to high growth rates and phlorotannin content. Growth of some Laminariales has been correlated with the season of peak inorganic nitrogen concentrations (reviewed by Dunton and Dayton 1995), which may be due to their ability to store carbon reserves from summer for use in growth when nitrogen conditions are replete (Gagné et al. 1982).

The results from the current study do not strongly support the hypothesis that a negative correlation exists between kelp phlorotannin content in and *L. vincta* density on the four studied kelp species within Kachemak Bay. This conclusion indicates that variables other than or in addition to phlorotannins may influence *L. vincta* density, distribution and feeding on kelp tissues. The prediction that phlorotannin content is higher in kelp tissues during the summer than the winter was only substantiated by data from one kelp species, the annual canopy-former *N. luetkeana*. Since the perennial understory species showed an inverse seasonal pattern of phlorotannin content as compared to that of *N. luetkeana*, life history strategy seems to have a dominant influence on phlorotannin regulation. The significance of environmental variables on phlorotannins differed between the species, and was also opposite between annual and perennial species. In summary, it seems likely that within the context of life history strategies, complex

interactions of physical factors including light and nutrient dynamics ultimately dictate phlorotannin content in kelp species.

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Table 2.1 Regression equations ($y = m$ (μg standard) + absorbance) fitted to phlorotannin standard curve specific to each kelp species (n=10). Range of phlorotannin standard masses used was 0-500 μg, with the linear portion of curves occurring from 0-250 μg. Standard error of slopes and R^2 values for all replicates of each species are shown.

kelp species	regression equation	slope SE	R^2
N. luetkeana	y = 0.00041x + 0.006	0.00002	0.991
A. clathratum	y = 0.00265x + 0.029	0.00050	0.995
S. latissima	y = 0.00011x + 0.005	0.00001	0.995
S. subsimplex	y = 0.00023x + 0.006	0.00002	0.989

Table 2.2 Results of two-factor ANOVAs on ranks of normal scores of *L. vincta* density on *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* stipe and whole blade tissue during summer and winter at Hesketh Island and Jakolof Bay.

	Hesketh Island			Jakolof Bay		
Source	*df*	*F*	*p*	*df*	*F*	*p*
Stipe tissue						
season	1	0.90	0.34	1	0.34	0.56
kelp species	3	17.7	<0.01	3	17.9	<0.01
season x kelp species	3	3.01	0.04	3	0.73	0.54
error	80			48		
Whole blade tissue						
season	1	12.6	<0.01	1	18.4	<0.01
kelp species	3	0.44	0.72	3	1.01	0.40
season x kelp species	3	7.22	<0.01	3	3.96	0.01
error	86			48		

Table 2.3 Results of two-factor ANOVAs on $\sin^{-1}(\sqrt{\ })$-transformed phlorotannin content in *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* meristematic stipe and whole blade tissue during summer and winter at Hesketh Island and Jakolof Bay.

	Hesketh Island			Jakolof Bay		
Source	*df*	*F*	*p*	*df*	*F*	*p*
Meristematic stipe tissue						
season	1	0.07	0.79	1	1.08	0.30
kelp species	3	475	<0.01	3	187	<0.01
season x kelp species	3	0.75	0.52	3	1.49	0.22
error	80			48		
Whole blade tissue						
season	1	5.51	0.02	1	1.22	0.27
kelp species	3	60.0	<0.01	3	9.07	<0.01
season x kelp species	3	13.9	<0.01	3	1.23	0.30
error	86			48		

Table 2.4 Environmental variables (light attenuation (% of surface irradiance at 1 or 5m), nitrate, ammonium, phosphate, silicate (each μM); mean ± 1 SE) measured during summer and winter. Data are grouped for both sites based on insignificant differences of each factor between sites. Sample sizes are shown for each season. Significant differences in a variable between summer and winter are denoted by *** ($p < 0.001$).

season	light (%)	nitrate (μM) ***	ammonium (μM)	phosphate (μM) ***	silicate (μM) ***	*n*
summer	39.8 ± 6.48 (1m) 79.9 ± 3.27 (5m)	1.69 ± 0.31	0.59 ± 0.10	0.61 ± 0.03	5.82 ± 0.56	15 (light) 21 (nuts.)
winter	38.6 ± 13.4 (1 m) 58.2 ± 10.4 (5 m)	12.3 ± 0.53	0.31 ± 0.12	1.37 ± 0.04	23.9 ± 1.41	6 (light) 7 (nuts.)

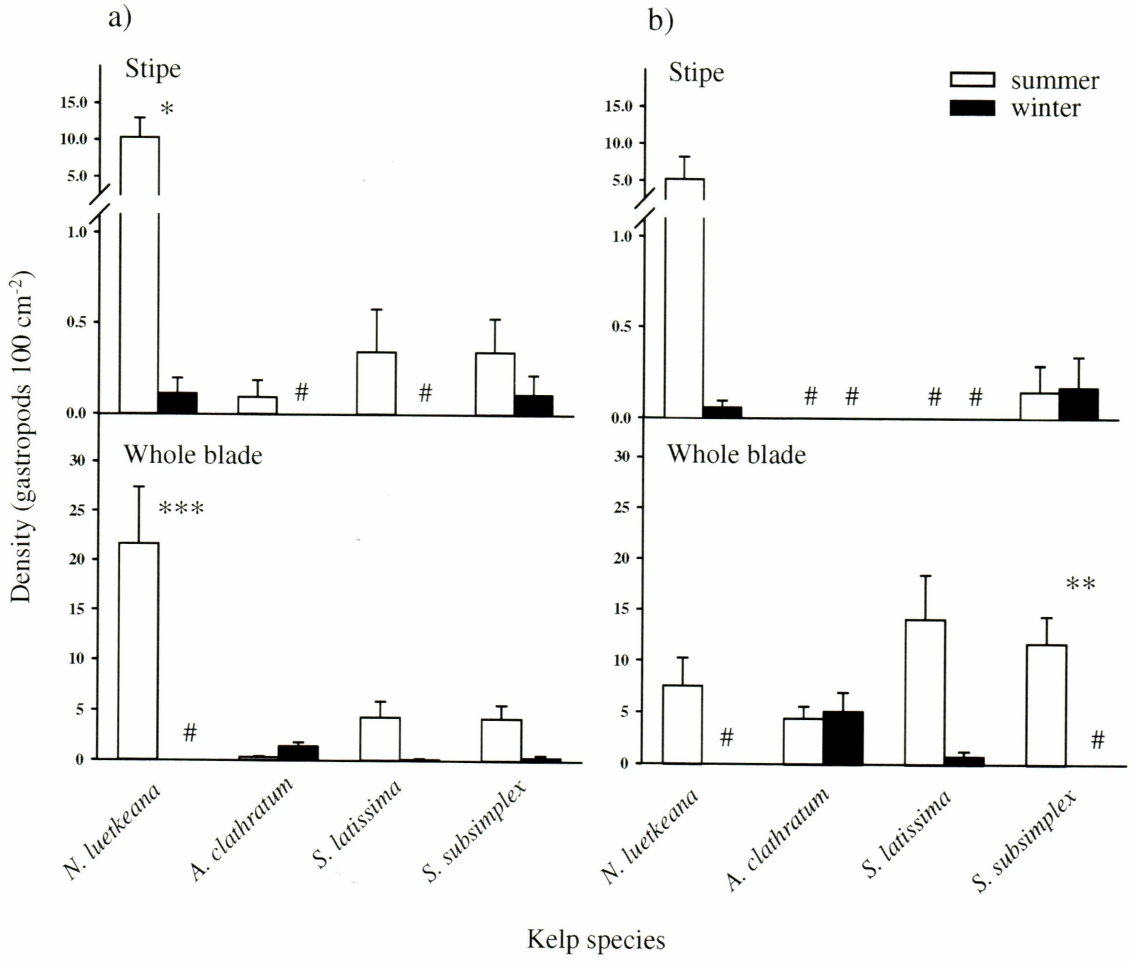


Fig. 2.1 *Lacuna vincta*. Density (gastropods 100 cm^{-2}; mean ± 1 SE) on stipe and whole blade tissue (n = 3-18 per tissue type) of *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* during the summer and winter at (a) Hesketh Island and (b) Jakolof Bay. The absence of *L. vincta* on stipe or blade tissue of particular kelp species during summer or winter is represented by #. Significant differences in density between seasons are denoted by * ($0.05 > p > 0.01$), ** ($0.01 > p > 0.001$), *** ($p < 0.001$). Note difference in y-axis scales between tissue types.

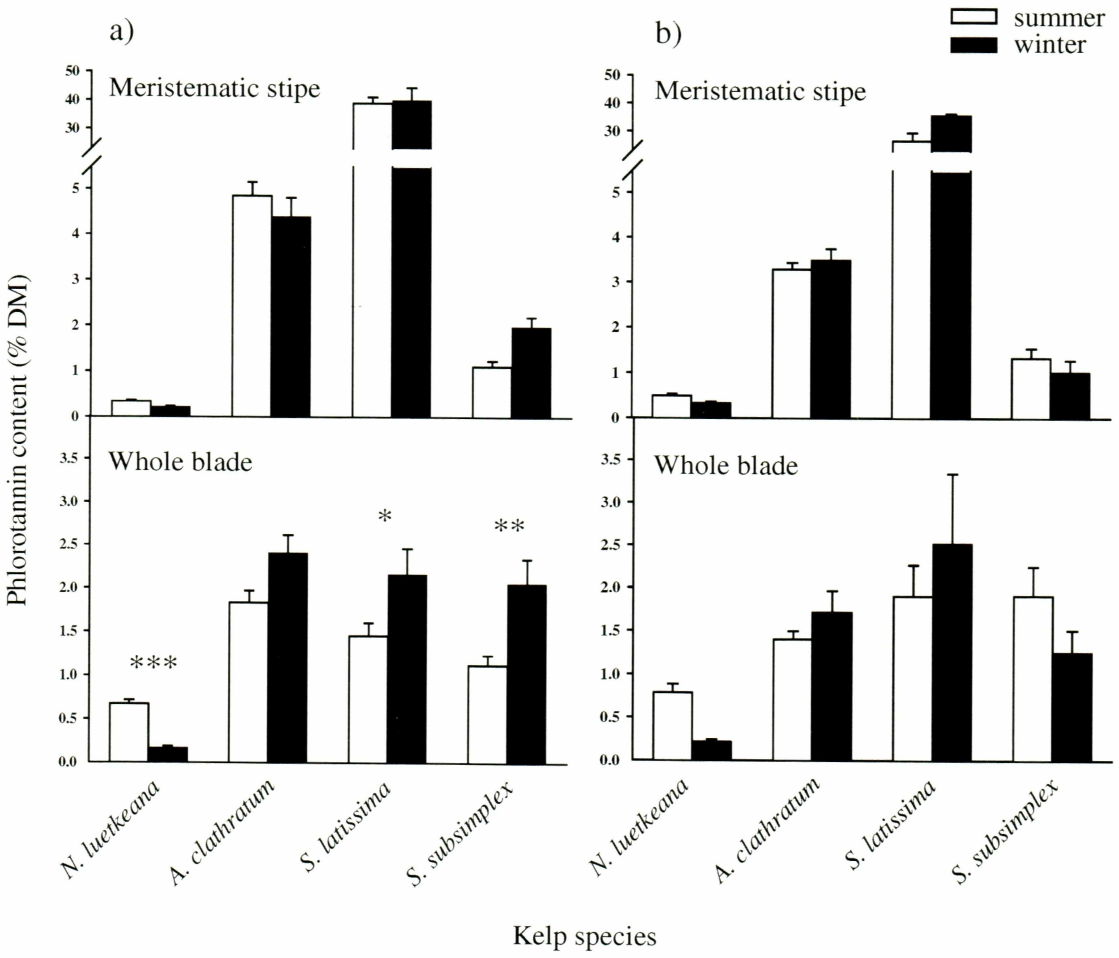


Fig. 2.2 *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex*. Phlorotannin content (% dry mass (DM); mean ± 1 SE) in meristematic stipe and whole blade tissue (n = 5-36 per tissue type) during the summer and winter at (a) Hesketh Island and (b) Jakolof Bay. Significant differences in phlorotannin content between seasons are denoted by * ($0.05 > p > 0.01$). Note difference in y-axis scales between tissue types.

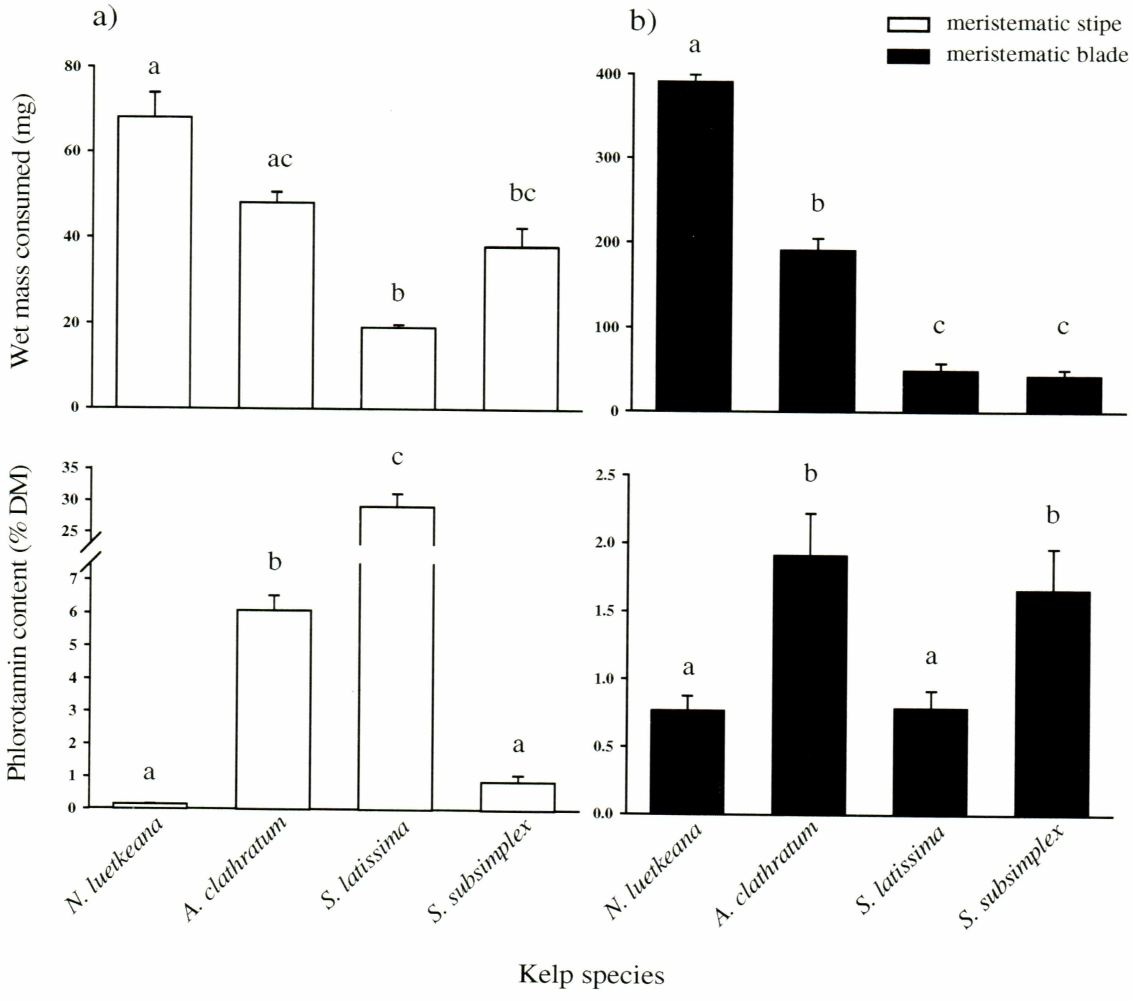
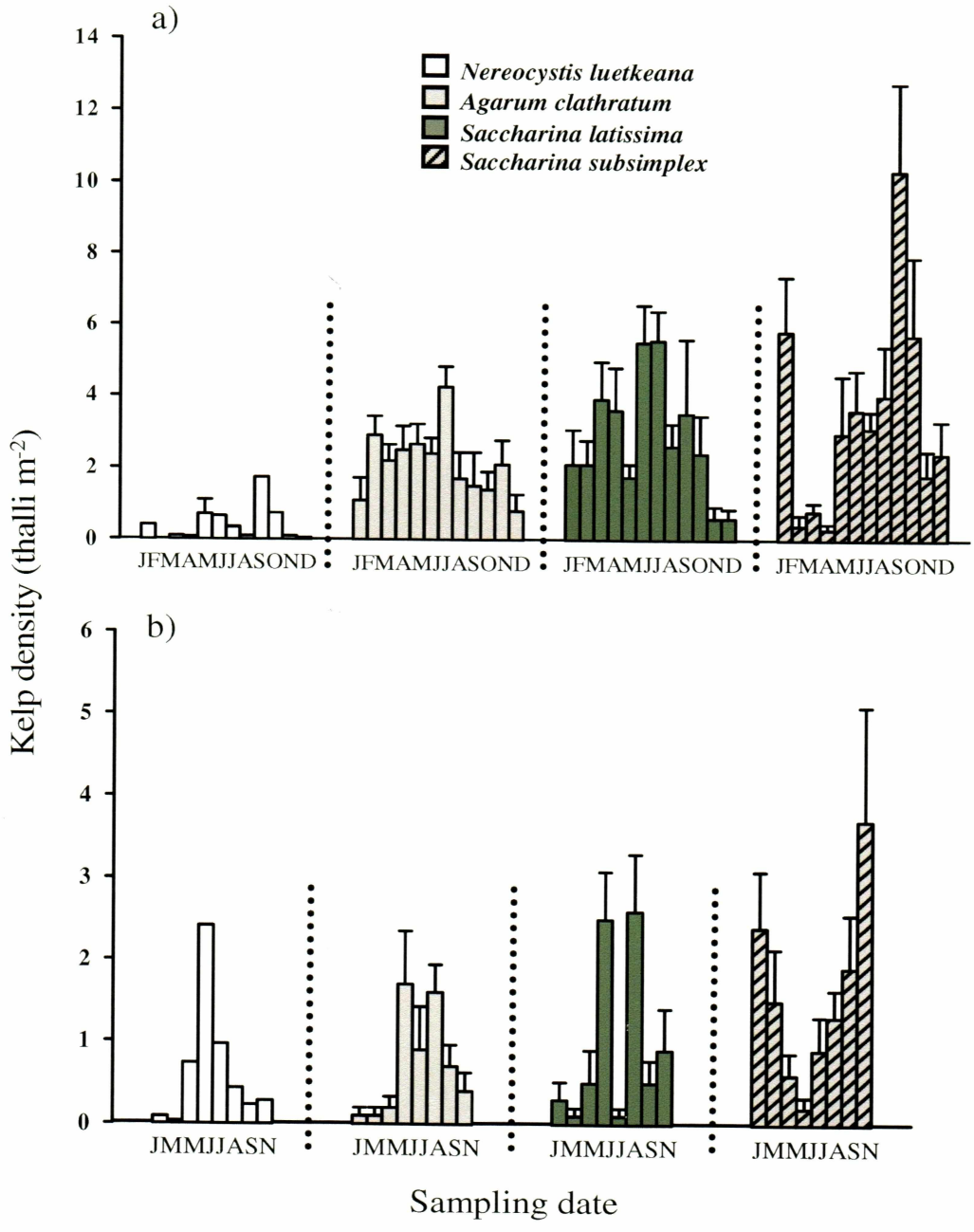
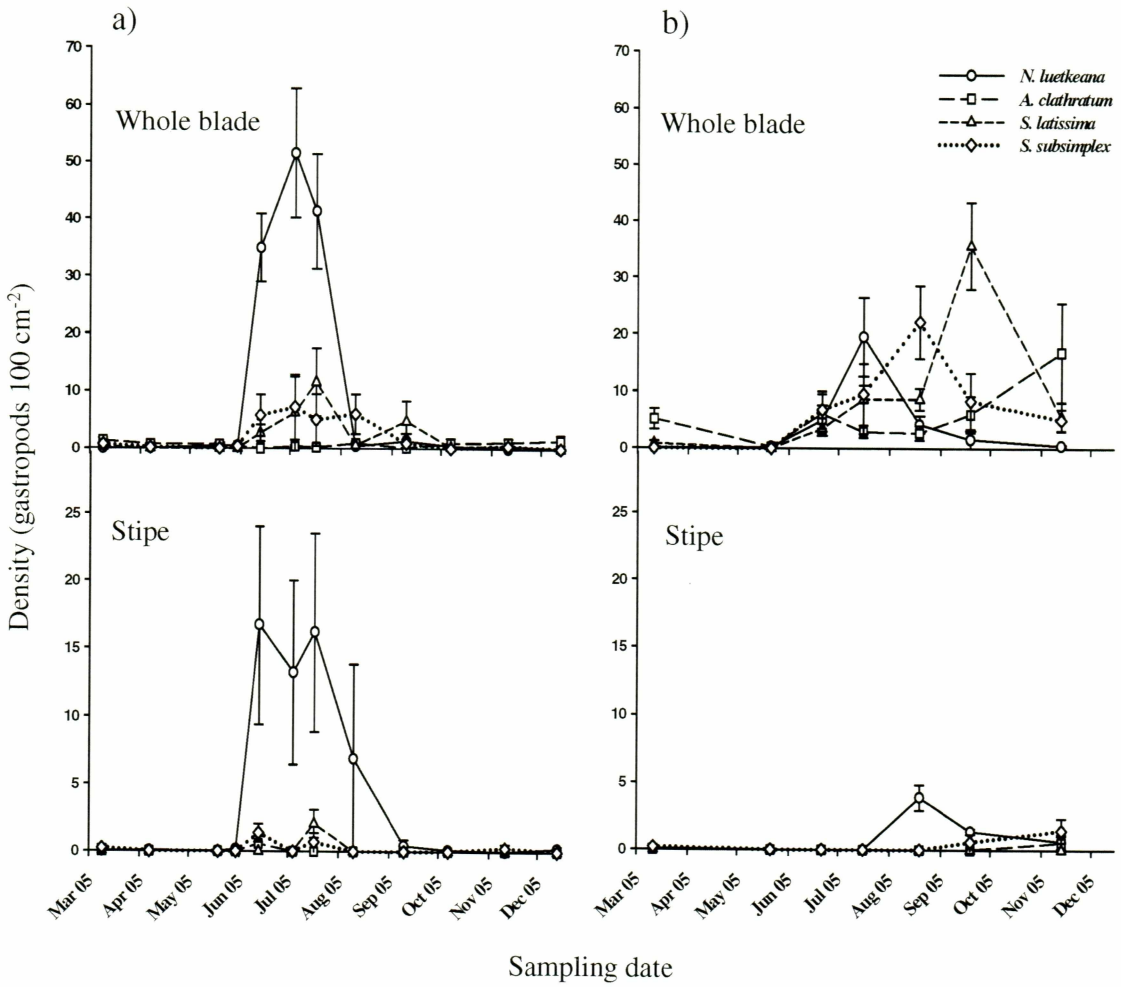


Fig. 2.3 *Lacuna vincta*. Wet mass consumed (mg; mean ± 1 SE) and phlorotannin content (% DM; mean ± 1 SE) of *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* (a) meristematic stipe and (b) meristematic blade tissue segments ($n = 10$ per tissue type). Within tissue types, bars with different letters are significantly different ($p < 0.05$). Note differences in y-axis scales between sites and tissue types.

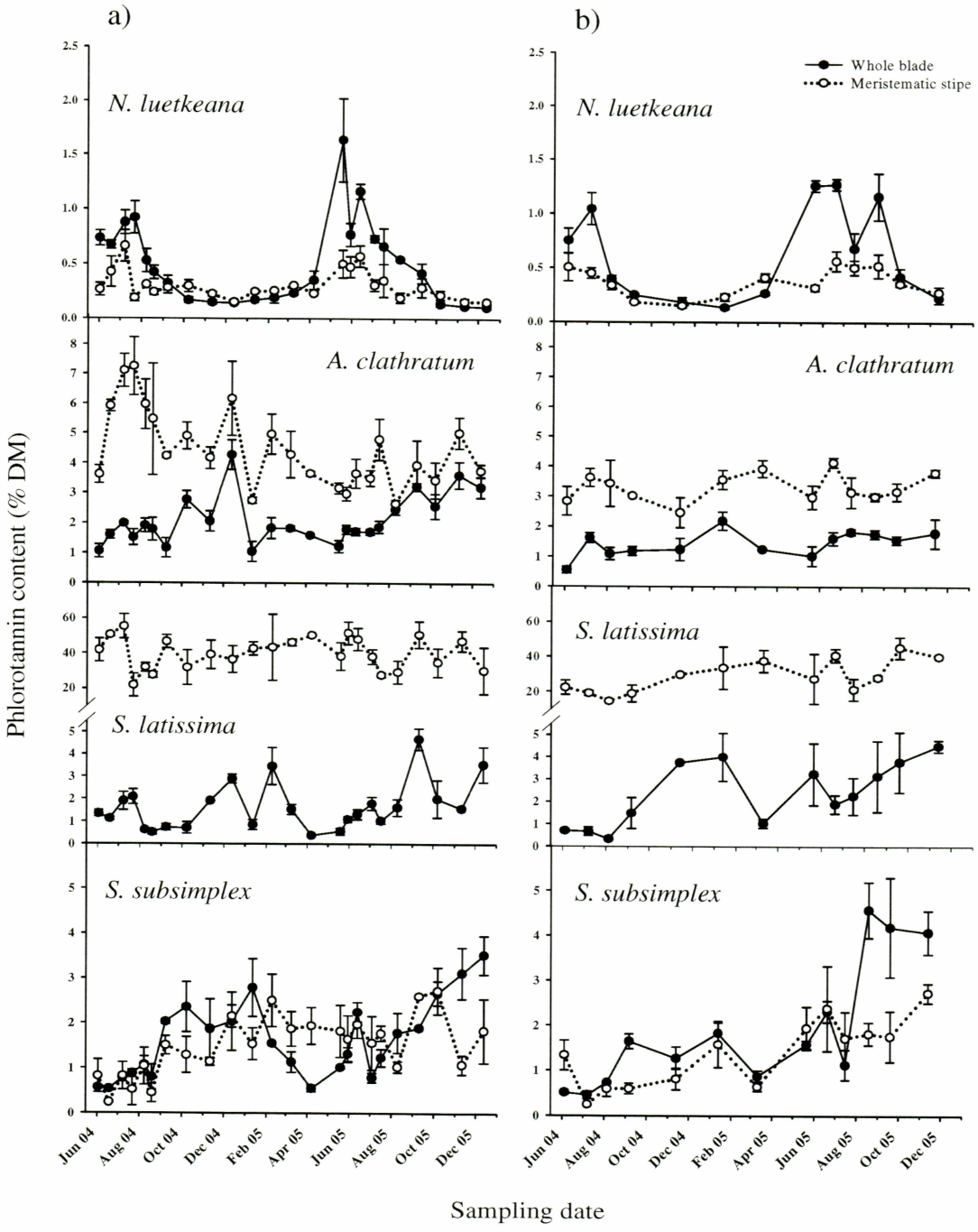
2.8 Appendices



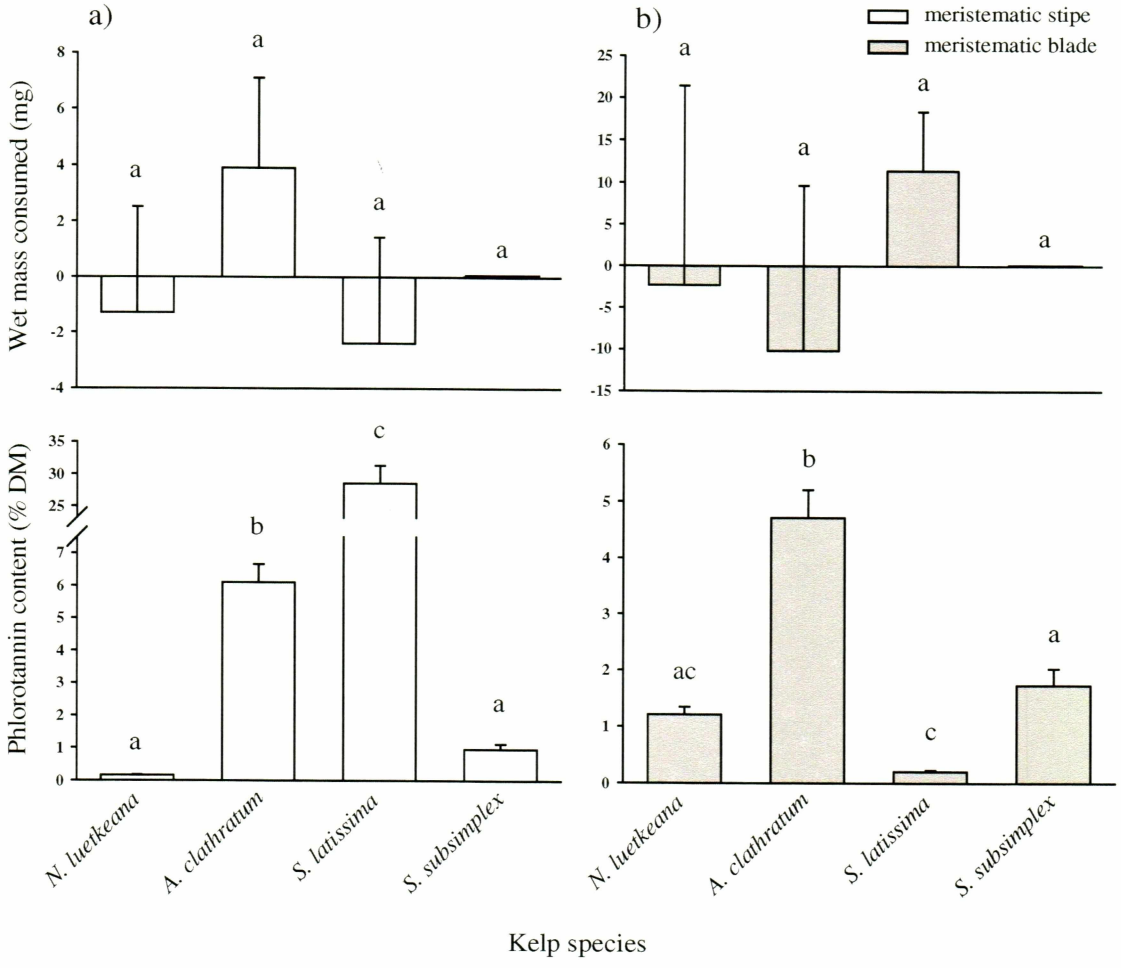
Appendix 2.A *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex*. Density (thalli m^{-2}; mean ± 1 SE) at (a) Hesketh Island (January-December 2005; n = 10-20 quadrats per sampling date) and (b) Jakolof Bay (January-November 2005; n = 10 quadrats per sampling date). Sample size (n = 1-2 per date) for *N. luetkeana* is based on use of swaths). Note difference in y-axes between sites.



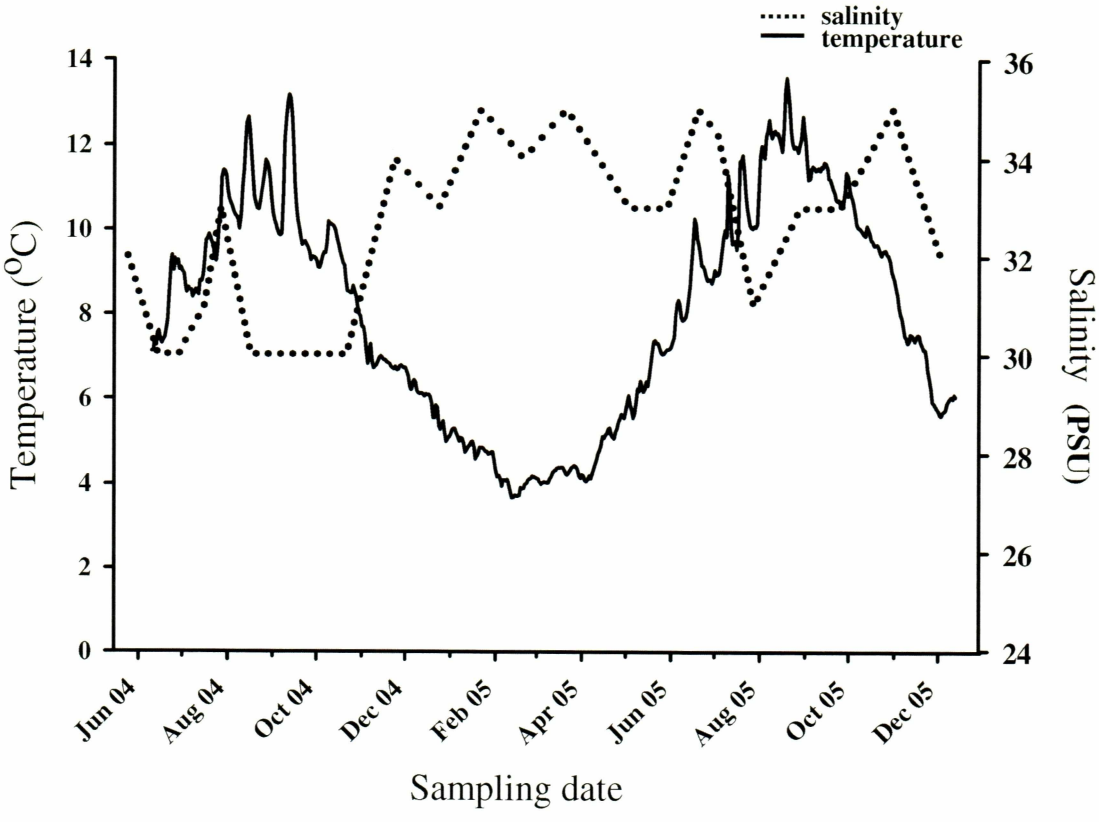
Appendix 2.B *Lacuna vincta*. Density (gastropods 100 cm^{-2}; mean ± 1 SE) on *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* whole blade or stipe tissue ($n = 3$ per tissue type) at (a) Hesketh Island (March-December 2005) and (b) Jakolof Bay (March–November 2005). Note difference in y-axes between tissue types.



Appendix 2.C *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex*. Phlorotannin content (% dry mass (DM); mean ± 1 SE) of whole blade and meristematic stipe tissue (n = 3 per tissue type) at (a) Hesketh Island (June 2004-December 2005) and (b) Jakolof Bay (June 2004-November 2005). Note differences in y-axes between species.



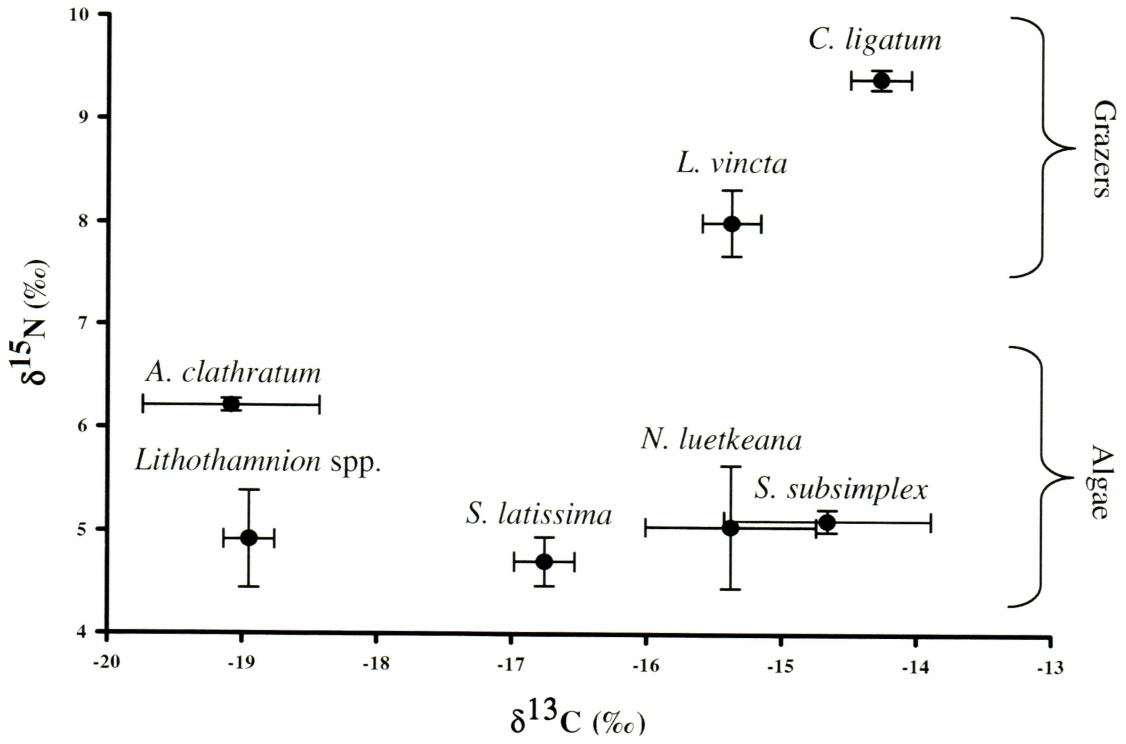
Appendix 2.D *Calliostoma ligatum*. Wet mass consumed (mg; mean ± 1 SE) and phlorotannin content (% DM; mean ± 1 SE) of *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* (a) meristematic stipe and (b) meristematic blade tissue segments (n = 10 per tissue type). Within tissue types, bars with different letters are significantly different ($p < 0.05$). Note differences in y-axis scales between sites and tissue types.



Appendix 2.E Temperature (°C; daily mean) and salinity (PSU; monthly or bimonthly mean) at 1 m above substrate at Hesketh Island.

Appendix 2.F *Lithothamnion* spp. (calcareous red algae), *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima*, *S. subsimplex* (kelps), *Calliostoma ligatum*, *Lacuna vincta* (grazers). Tissue carbon and nitrogen content (%) and C:N (mean ± 1 SE; $n = 3$ per tissue type and $n = 5$ per grazer species).

Tissue origin	% C ± SE	% N ± SE	C:N ± SE
Algae			
Lithothamnion spp.	13.1 ± 0.88	2.10 ± 0.13	6.24 ± 0.15
Nereocystis luetkeana			
holdfast	28.6 ± 0.60	2.82 ± 0.42	10.5 ± 1.26
stipe	36.6 ± 0.68	3.27 ± 0.51	11.8 ± 1.93
blade	31.3 ± 0.83	2.95 ± 0.17	10.6 ± 0.43
Agarum clathratum			
holdfast	33.4 ± 3.78	2.60 ± 0.09	12.9 ± 1.07
stipe	48.4 ± 2.94	4.15 ± 0.43	11.8 ± 0.57
blade	37.6 ± 4.90	3.24 ± 0.48	11.7 ± 0.28
Saccharina latissima			
holdfast	35.0 ± 0.72	2.17 ± 0.14	16.2 ± 1.05
stipe	39.3 ± 2.72	2.47 ± 0.58	17.0 ± 2.57
blade	28.7 ± 4.65	1.61 ± 0.15	17.6 ± 1.39
Saccharina subsimplex			
holdfast	32.2 ± 2.71	2.99 ± 0.11	10.8 ± 0.77
stipe	43.6 ± 5.82	3.92 ± 0.80	11.9 ± 2.28
blade	42.5 ± 0.28	2.28 ± 0.23	19.0 ± 1.82
Gastropods			
Calliostoma ligatum	45.8 ± 1.25	12.8 ± 0.37	3.57 ± 0.02
Lacuna vincta	44.3 ± 1.29	10.8 ± 0.44	4.13 ± 0.19



Appendix 2.G *Lithothamnion* spp. (calcareous red algae), *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima*, *S. subsimplex* (kelps), *Calliostoma ligatum*, *Lacuna vincta* (grazers). $\delta^{13}C$ and $\delta^{15}N$ (‰) stable isotopes (mean ± 1 SE; $n = 3$ per algal species and $n = 5$ per grazer species). Isotope data presented for kelp species are from blade tissue only.

Chapter 3 - Within-thallus phlorotannin allocation and induction in Northeastern Pacific kelps[2]

3.1 Abstract

Phlorotannins are typical kelp chemical constituents often associated with antiherbivore properties. Within-thallus phlorotannin distribution is frequently explained by allocation theories like the Optimal Defense Theory (ODT) based on the relative value of tissue types and the metabolic cost of chemical defense production. In accordance with the ODT, we hypothesized that during the summer, attachment structures (holdfasts and stipes), meristematic blades and reproductive tissue from the canopy-forming *Nereocystis luetkeana* and understory kelps *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* from Kachemak Bay, Alaska, would contain higher phlorotannin content than vegetative tissues. Assuming feeding deterrent properties of phlorotannins, we expected a negative relationship between within-thallus distribution of the gastropod grazer *Lacuna vincta* and phlorotannin content. Allocation of phlorotannins to holdfast and/or stipe tissue and meristematic blades was higher than to vegetative tissues in all kelp species, though trends in reproductive tissue were inconsistent between species. The adherence of within-thallus phlorotannin distribution to the model proposed by the ODT may confirm the relative fitness values of selected tissue types within the four kelp species. Within thalli, phlorotannin content and *L. vincta* density were not consistently negatively correlated, indicating that phlorotannins may not cause deterrence in this grazer and/or serve another purpose in these kelp species. Phlorotannin production may also be induced in the event of sudden, unpredictable grazing damage. We hypothesized that mechanical damage to meristematic tissue of *A. clathratum* and *S. latissima* would induce phlorotannin production since loss of this tissue type to grazing may affect growth and photosynthetic and reproductive potential. This hypothesis was not supported, which may suggest that a constitutive defense strategy is most efficient in the study environment.

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[2] Dubois, A. and K. Iken. 2006. Within-thallus phlorotannin allocation and induction in Northeastern Pacific kelps. Submitted to Journal of Experimental Marine Biology and Ecology.

Key words: induction; kelp; *Lacuna vincta* distribution; Optimal Defense Theory; within-thallus phlorotannin allocation

3.2 Introduction

Kelps (Order Laminariales) possess differentiated tissues with particular roles in the function and life history of the individual (Cronin and Hay, 1996). Thalli are usually structured into distinct holdfast, stipe and blade tissues, sometimes pneumatocysts for flotation, and defined reproductive regions. These kelp tissue types often contain phlorotannins, phloroglucinol-based monomers present in most brown algae (Ragan and Glombitza, 1986). Phlorotannins have been identified as having primary metabolic roles in wound healing (Lüder and Clayton, 2004) and cell wall construction (Schoenwaelder and Clayton, 1999). Also, they have been assigned secondary functions such as protection against UV radiation (Pavia et al., 1997), larval settlement, bacterial and fungal growth (Ragan and Glombitza, 1986; Wikström and Pavia, 2004), and grazing damage (for reviews see Targett and Arnold, 1998; Amsler and Fairhead, 2006).

Differential allocation of phlorotannins within brown algal tissues has been shown for several temperate and polar species (Van Alstyne et al., 1999; Amsler and Fairhead, 2006). Patterns of phlorotannin allocation are often related to a variety of ecological theories, most frequently the Optimal Defense Theory (ODT) (see Amsler and Fairhead, 2006 for comprehensive review). The ODT presumes that defenses are metabolically costly and therefore not constantly produced in high concentrations throughout all kelp tissues. Thus, defenses should be allocated more to tissue types that are of greater importance to individual fitness and are most likely to be attacked by grazers. The tissue types that are generally considered most vital for kelps are those necessary for substrate attachment, growth and reproduction, with vegetative tissues being of lesser importance (Van Alstyne et al., 1999). This assignment of relative significance of tissues has been supported in some studies based on within-individual phlorotannin comparisons of brown algae (e.g. Johnson and Mann, 1986; Van Alstyne et al., 1999; Pavia et al., 2002).

Phlorotannin production and cost in kelps can also be considered in the context of constitutive and inducible defense strategies (Steinberg, 1994; Hammerstrom et al., 1998). Constitutive defenses are continuously produced at relatively unchanging concentrations and are typically observed in environments where grazing is constant and/or predictable (Herms and Mattson, 1992). Inducible defenses are typically activated through particular mechanical signals such as tissue damage by grazers (Harvell, 1990; Herms and Mattson, 1992). They are most beneficial in environments where herbivorous damage is variable over time and/or is unpredictable (Harvell, 1990). Induction may also be favored where the predominant grazers are small and relatively immobile (Hay, 1996) and when the response to herbivorous damage is rapid and grazer deterrence is effective (Herms and Mattson, 1992; Toth et al., 2005). Single wounding events simulating herbivory have yielded mixed results, often not causing induced responses in kelp blade tissue (Steinberg, 1994, 1995; Martinez, 1996; Toth and Pavia, 2002a). However, rapid induction (within 1-3 days after wounding) of phlorotannins was observed in four of five kelp species in the Northeast Pacific San Juan Islands (Hammerstrom et al., 1998) and microscopically in Australian *Ecklonia radiata* (Lüder and Clayton, 2004). Studies utilizing single wounding simulations with Fucalean algae have also induced (Van Alstyne, 1988; Yates and Peckol, 1993; Hemmi et al., 2004) or not induced (Steinberg, 1994; Pavia et al., 1997) phlorotannin responses. Repeated wounding experiments test the potential magnitude and duration of induced responses simulating continued feeding of grazers. Since phlorotannins may not be immediately effective in deterring feeding by some grazers (Van Alstyne, 1988), knowledge of the effects of repeated wounding on induction is important to the study of kelp defensive chemical strategies. To our knowledge, no repeated wounding experiments have been conducted on kelp.

Gastropods can be abundant kelp grazers and, among other factors, the tissue-specific presence of chemical defenses may influence herbivore distribution within a kelp thallus or among species (reviewed by Paul et al., 2001). The dominant grazer in the study environment of Kachemak Bay, Alaska, is the mesogastropod *Lacuna vincta* (Montagu), which is seasonally abundant in the shallow subtidal zone and can reach

densities of 70 snails 100 cm^{-2} in summer (see Chapter 2). *Lacuna vincta* is a voracious grazer within kelp canopies (Duggins et al., 2001; Chenelot, 2003; Carney et al., 2005; see Chapter 2) and is capable of decimating kelp blade tissue (Johnson and Mann, 1986). Given the seasonal risk of grazing attack by *L. vincta* on kelps in the study environment, we sought to test the predictions of the ODT for phlorotannin allocation within kelp tissues. Based on the framework of this theory, we hypothesized that phlorotannins would be allocated differentially to distinct tissue types within *Nereocystis luetkeana* (Mertens) Postels et Ruprecht, *Agarum clathratum* Dumortier, *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (formerly *Laminaria saccharina*) and *S. subsimplex* (Setchell & N.L. Gardner) Widdowson, S.C. Lindstrom & P.W. Gabrielsen (formerly *L. bongardiana*) thalli. Since holdfasts and stipes are vital to overwintering in understory perennials and to substrate attachment in all species and meristematic and reproductive blade tissues are essential for growth and spore production, respectively, we predicted that these tissue types would contain higher phlorotannin content than non-meristematic and degenerating blades. Given the assumed feeding deterrent effects of phlorotannins, we also hypothesized that spatial distribution of *L. vincta* on kelp thalli would be negatively related to the distribution of phlorotannins within thallus tissue types. Lastly, we assessed the phlorotannin induction potential of meristematic blade tissue of the two understory species, *A. clathratum* and *S. latissima*. We hypothesized that induction would occur based on mechanical wounding simulating herbivory by *L. vincta* since damage to meristematic blade tissue may be detrimental to individual growth as well as photosynthetic and reproductive potential.

3.3 Materials and Methods

3.3.1 Kelp tissue and *Lacuna vincta* sampling

Sample sites were located near the western end of Hesketh Island (59° 30.3' N, 151° 31.8' W) and at the mouth of Jakolof Bay (59° 28.1' N, 151° 32.0' W) within Kachemak Bay on the south-central coast of Alaska (see Chapter 1). The study was

replicated at the two sites to establish whether observed trends were consistent and independent of location. Study areas were typically 4 m below mean low water neap tide levels (MLWN) and were chosen based on similar physical factors such as wave exposure, temperature, light availability, salinity and nutrient regime, and biological factors like grazer and algal assemblages.

Semimonthly observations of grazer density and distribution (August and September 2004 and June through September 2005) and collections of kelp tissues (June through September 2004 and 2005) were conducted at Hesketh Island. Grazer density and distribution on *N. luetkeana* tissues was determined only during 2005. Due to logistical constraints, sampling of all kelp species at Jakolof Bay occurred half as frequently. Field investigations occurred along one 30 m transect placed randomly within the kelp bed at each study site. Three replicate thalli of the understory kelp species *A. clathratum*, *S. latissima* and *S. subsimplex* and the canopy-forming species *N. luetkeana* were selected randomly along the transect, removed from the substrate below the holdfast and kept in cold seawater in the dark for transport. In the lab, stipe length and circumference and blade length and width were measured for approximate surface area. Circumference of the pneumatocyst was also determined for *N. luetkeana* thalli. Surface area estimates were not made for holdfast and reproductive tissue in any species. A segment (<1.5 g) of each tissue type was then excised from all replicates for measurement of phlorotannin content. Holdfast tissue samples consisted of at least two distinct haptera per individual. Stipe tissue was taken from the meristematic region just above the holdfast. Blade pieces were removed from the lower portion of each tissue type (meristematic, non-meristematic and degenerating blade) just to the side of the midrib (*A. clathratum*) or longitudinal axis (*N. luetkeana*, *S. latissima*, *S. subsimplex*). Reproductive tissue was taken when available. All tissue was kept frozen in the dark until phlorotannin analysis.

Lacuna vincta density was quantified separately on the holdfast, stipe (including pneumatocyst for *N. luetkeana*), and meristematic, non-meristematic and degenerating blade portions of the same three replicate thalli per kelp species before they were sampled for phlorotannin analysis. Number of *L. vincta* per unit area was determined based on the

amount of snails on each tissue type given the total surface area of that tissue region. Total *L. vincta* density on *N. luetkeana* blades was determined by counting all individuals on 3 blades and extrapolating based on the total number of blades per thallus. Due to the inherent patchy distribution of grazers, some sampling error likely resulted from this method of *L. vincta* quantification.

3.3.2 Kelp phlorotannin analysis

Phlorotannins were quantified using the 2, 4-dimethylbenzaldehyde (DMBA) method (Stern et al., 1996). Extraction of kelp tissue for species-specific standards was conducted as described in Stern et al. (1996; also see Chapter 2 for details). Briefly, ground kelp tissue was extracted in 80% methanol, the filtrate refined with toluene and acetone, dried under reduced pressure, and the phlorotannin yield freeze-dried for 48 h and kept frozen until analyzed. A standard curve was determined for each kelp species using 0-500 $\mu g\ \mu L^{-1}$ phlorotannin mass to methanol volume. Standard curve slopes were used in the calculation of tissue phlorotannin content expressed as % dry mass (DM) for each species (see Chapter 2). Tissue phlorotannin content was measured spectrophotometrically at 510 nm (Thermospectronic Genesys 5) from methanolic extracts as described by Stern et al. (1996). Wet:dry mass for each tissue type per kelp species ($n = 6$) was determined after drying at 60 °C for 48 h.

3.3.3 Induction experiment

Agarum clathratum and *S. latissima* were used in an induction study that assessed the effects of mechanical damage simulating grazing such as that caused by *L. vincta* on meristematic blade phlorotannin content. A pilot study conducted in March of 2005 determined that 10 *A. clathratum* thalli (effect size = 1.49; $\alpha = 0.05$) and 8 *S. latissima* thalli (effect size = 1.55; $\alpha = 0.05$) would be used in order to achieve a power of 0.85 given typical intraspecies variability in phlorotannin content.

Induction studies were conducted *in situ* adjacent to the Hesketh Island and Jakolof Bay sites from June 5-12 and July 1-8 of 2005, respectively. One 30 m permanent

transect was established at each location, along which treatment and control thalli of both species were marked and sampled daily at the same time for 7 days. A first set of treatment thalli (n = 8 or 10 per species as mentioned above) was repeatedly wounded by removing a 1 cm diameter piece of meristematic tissue approximately 5 cm above the blade base with a cork borer every 24 hours (Fig. 3.1a). The excised tissue also served as a sample for phlorotannin analysis. The tissue punch made each consecutive day was located adjacent to the previous punch for an overall concentric pattern. A second treatment type accounted for kelp responses to a single wounding event and involved punching previously undisturbed thalli (n = 8 or 10 per species) once on the first and again on the seventh day (Fig. 3.1b). A daily baseline control consisted of a single punch on previously undisturbed thalli (n = 8 or 10 per species; Fig. 3.1c) that were located in the same region along the transect as the treatment thalli. This baseline control established typical daily phlorotannin content in the absence of prior mechanical wounding by the cork borer. A second control accounted for small-scale variability in phlorotannin content within a localized area of the meristematic blade. On previously unsampled individuals (n = 8 or 10 per species), 5 adjacent punches were sampled once on the first day of the induction study (Fig. 3.1d). For all treatments and controls, each tissue piece was kept separately in cold seawater in the dark for transport.

In the lab, excised wet tissue was weighed and frozen for later phlorotannin analysis. Measurements of phlorotannin content elucidated differences between the repeated wounding treatment and the baseline control as well as the single wounding treatment and the baseline control over the 7 day study duration. Results were then considered with respect to the magnitude of localized variability in phlorotannin distribution within a small tissue area.

3.3.4 Statistical analysis

Prior to analysis, all data were assessed for normality and equal variances using Shapiro-Wilk tests (Zar, 1999) and residual plots. Phlorotannin content data were arcsine-square root transformed to improve error variance and *Lacuna vincta* density data were

left untransformed before use of repeated-measures analyses of variance (ANOVA) with tissue type as the repeated measure. Phlorotannin content analyses included five tissue types (holdfast, meristematic stipe, meristematic blade, non-meristematic blade and degenerating blade) and *L. vincta* density four tissue types (entire stipe, meristematic blade, non-meristematic blade and degenerating blade). ANOVA were performed separately for phlorotannin content and *L. vincta* density at each site and for each kelp species. When the sphericity assumption associated with univariate analyses of within-subject effects was violated, the MANOVA Wilks' Lambda test statistic was used. Planned contrasts evaluated significant differences between tissue types in each of the analyses. Repeated-measures ANOVA were also used on raw ratio data of *L. vincta* density to phlorotannin content. These analyses determined differences in the relationship between within-thallus snail density on, and phlorotannin content in, four tissue types (stipe and three blade types) of each kelp species. Pearson's product-moment correlations were used to assess the whole-thallus relationship between *L. vincta* distribution and phlorotannin content of each kelp species, such that all tissue types per individual were grouped for the analyses.

Analyses on untransformed phlorotannin induction data were conducted using repeated-measures ANOVA separately for each kelp species and site with time as the repeated measure. Within-subject effects were determined based on the difference in phlorotannin content between repeatedly wounded treatment and paired baseline control thalli on each day of the experiment. Planned comparisons related mean differences on day n to the initial phlorotannin difference at day 0. The differences between the single-wounding treatment thalli and baseline control thalli on day 7 versus day 0 were assessed using a paired t-test on raw data. All analyses were conducted using SAS v9.1 software (SAS Inc., Cary, NC) at $\alpha = 0.05$.

3.4 Results

3.4.1 Kelp phlorotannin content and *Lacuna vincta* density

Phlorotannin content data demonstrated significant differences with respect to tissue type in *N. luetkeana*, *A. clathratum*, *S. latissima* and *S. subsimplex* at Hesketh Island and Jakolof Bay (each $p < 0.001$; Table 3.1, Fig. 3.2). Phlorotannin allocation patterns within thalli were not consistent between kelp species (Fig. 3.2). *Nereocystis luetkeana* and *S. subsimplex* had higher phlorotannin content in holdfast than meristematic stipe tissue at both sites (each $p < 0.05$ except for *S. subsimplex* at Jakolof Bay), while *A. clathratum* and *S. latissima* demonstrated the opposite trend (each $p < 0.05$ except for *A. clathratum* at Jakolof Bay). The high phlorotannin content in *S. latissima* holdfast and stipe tissues was unique amongst the studied kelps. Within blade tissue types, the meristematic region accounted for the highest phlorotannin content in *N. luetkeana*, *A. clathratum* and *S. latissima* at both sites (each $p < 0.05$). *Saccharina subsimplex* had similarly high phlorotannins in meristematic and non-meristematic blade tissues, which were significantly greater than degenerating blade phlorotannins at both sites (each $p < 0.05$). A general decreasing pattern of phlorotannins from the meristematic base to the degenerating tips of blades was evident for all kelp species at both sites (Fig. 3.2). When present, reproductive tissue in *N. luetkeana* demonstrated variable phlorotannin values (0.35 ± 0.01 % DM; $n = 3$ at Hesketh Island and 0.64 ± 0.01 % DM; $n = 5$ at Jakolof Bay), though reproductive tissue phlorotannin content was consistently lower than in meristematic blade tissue. *Agarum clathratum* reproductive tissue contained 3.23 ± 0.21 % DM phlorotannins ($n = 10$) at Hesketh Island and 2.52 ± 0.29 % DM phlorotannins ($n = 7$) at Jakolof Bay. Allocation of phlorotannins to reproductive tissue in *A. clathratum* was comparable to that measured in meristematic blade tissue. Reproductive tissue was not observed in *S. latissima* or *S. subsimplex* during the study period.

Lacuna vincta was absent from all holdfast tissue and therefore this tissue type was not included in statistical analyses. Only on *N. luetkeana* stipe tissue did *L. vincta* reside in similar densities as on other tissue types (Fig. 3.3). The minimal abundance

observed on stipes of understory species relative to blade tissues is reflected in significant (or near significant in the case of *S. latissima* at Hesketh Island) tissue type effects for *A. clathratum*, *S. latissima* and *S. subsimplex* at both study sites (Table 3.1, Fig. 3.3). Apart from this, *L. vincta* was not clearly denser on a particular blade tissue type of any kelp species at either site (Fig. 3.3).

Ratios of *L. vincta* density to phlorotannin content in kelp thalli of each species revealed significant tissue type differences within *A. clathratum* ($p = 0.004$ and 0.012) and *S. subsimplex* ($p = 0.012$ and 0.009) at Hesketh Island and Jakolof Bay, respectively (Table 3.2). The effect of tissue type in *S. latissima* was significant at Jakolof Bay ($p = 0.009$) and nearly significant at Hesketh Island ($p = 0.052$; Table 3.2). The high ratio values observed for *N. luetkeana* tissues, especially at Hesketh Island and on stipe, non-meristematic and degenerating blade tissue, indicate high *L. vincta* density relative to low phlorotannin content (Fig. 3.4). Low ratio values shown for *A. clathratum* tissues and stipe, meristematic blade and non-meristematic blades of *S. latissima* and *S. subsimplex* demonstrate low *L. vincta* density on tissues containing relatively high phlorotannin content. With the exception of *N. luetkeana*, ratio values at Hesketh Island increased significantly ($p < 0.05$) from the bases to the tips of thalli, though differences between meristematic and non-meristematic blade tissue were not significant. The same increasing trend was not apparent at Jakolof Bay and ratio values were generally not different between blade tissue types (Fig. 3.4).

Correlations between *L. vincta* density and phlorotannin content within kelp thalli of each species demonstrated significant negative relationships in *A. clathratum* ($p = 0.004$) and *S. latissima* ($p < 0.001$) at Hesketh Island and *S. latissima* ($p < 0.001$) at Jakolof Bay (Table 3.3). Weak positive relationships occurred in *N. luetkeana* ($p = 0.029$) and *S. subsimplex* ($p = 0.049$) thalli at Jakolof Bay. Since phlorotannin content in tissue types of each kelp species was relatively consistent at both sites throughout the study period, differences in the direction of relationships between sites were likely a result of dissimilar *L. vincta* distribution (Table 3.3).

3.4.2 Induction experiment

In situ induction studies did not demonstrate an induced response within repeatedly wounded *A. clathratum* or *S. latissima* thalli at Hesketh Island (Table 3.4, Fig. 3.5). The daily differences in phlorotannin content between the repeatedly wounded and baseline control thalli at this site were similar and not significant (Fig. 3.5). Significant within-subject differences in phlorotannin content in both species at Jakolof Bay were likely caused by differences in the relationship between repeated wounding treatment and baseline control thalli on day 0 as compared to other days (Table 3.4, Fig. 3.6). Nevertheless, Jakolof Bay thalli did not clearly demonstrate an induced response based on repeated wounding (Fig. 3.6). The only significant difference from the single-wounding experiment was in *A. clathratum* at Jakolof Bay ($t_9 = -3.04$, $p = 0.014$) (Fig. 3.6). This statistical significance likely resulted from a large difference between single wounding treatment and baseline control thalli on day 7 as compared to day 0. Significance in this case does not seem to indicate a negative response in *A. clathratum*.

The control that accounted for localized phlorotannin content variability in meristematic blade tissue demonstrated considerable differences within and between individuals of both species. Within-individual phlorotannin content at Hesketh Island varied up to 3.06 % DM in *A. clathratum* and up to 4.78 % DM in *S. latissima*. Between-individual variability was slightly lower than within-individual variability at Hesketh Island in both species, at 2.19 % DM and 4.63 % DM in *A. clathratum* and *S. latissima*, respectively. Phlorotannin content variability within individuals of both species was slightly higher at Hesketh Island than at Jakolof Bay, though the same pattern of greater within-individual as opposed to between-individual variability was noted at both sites.

3.5 Discussion

The higher phlorotannin content in attachment structures and meristematic tissue as opposed to non-meristematic tissue in our study species supports our main hypothesis. Assuming that phlorotannins have ecological functions that improve the fitness of an individual, our results are in accordance with the predictions of the ODT based on

presumed high fitness value of these tissue types. In macrophytes possessing a single holdfast and stipe that connects all blade and reproductive tissue to the substrate, such as many Laminariales and Fucales, attachment structures can be viewed as highly important (Amsler and Fairhead, 2006). In addition, some perennial *Saccharina* (formerly *Laminaria*) spp. may over-winter as holdfasts and stipes (O'Clair and Lindstrom, 2000) and thus attachment structures may facilitate production of a new blade in the spring. Within blade tissues, the meristematic tissue can be assumed to be especially valuable for kelps because it is the main growing region of the thallus. Protection of meristematic blades relative to other blade tissues may be even more important in rapidly-growing annual species like *N. luetkeana* that have a limited season in which to produce photosynthetic tissue. Furthermore, blades of *N. luetkeana* are thin and narrow at their origin near the pneumatocyst, and therefore may be easy for grazers to damage and detach. Similar to our results, other studies of brown algae have noted a higher phlorotannin content in attachment and meristematic tissues than in non-meristematic tissues (Tugwell and Branch, 1989; Van Alstyne et al., 1999; Taylor et al., 2002; Fairhead et al., 2005; Toth et al., 2005). Additionally, Pavia et al. (2002) predicted and observed decreasing phlorotannin content from stipe to annual shoots to receptacles in *Ascophyllum nodosum*.

The ODT further predicts that the tissue types that are at the greatest risk of attack will be the most defended (Rhoades, 1979; Herms and Mattson, 1992). The establishment of a relative risk of attack to particular tissue types in kelp is difficult since all parts of the algal thallus are above the substrate and available to mobile grazers (Zangerl and Rutledge, 1996; Toth et al., 2005). However, some information may be inferred from a comparison between grazer and phlorotannin distributions within a kelp thallus. The relative absence of *L. vincta* on phlorotannin-rich holdfast and stipe tissue of all understory species may indicate some degree of deterrence by phlorotannins, though grazer distribution on tissue types within thalli was not clearly correlated to phlorotannin content in those respective tissues. Johnson and Mann (1986) attributed distribution of *L. vincta* on *Laminaria longicruris* (now *S. longicruris*) blade margins as compared to

intercalary meristems and attachment structures to varying concentrations of polyphenolic compounds as well as tissue toughness and nutritive value. In contrast, feeding preferences of *L. vincta* on *Laminaria hyperborea* fronds could not be related to phlorotannin content (Toth and Pavia, 2002b). In this study, the deterrent properties of phlorotannins against *L. vincta* have not been conclusively established (but see Johnson and Mann 1986 and Chapter 2 for correlative inferences). Furthermore, differences between study sites in *L. vincta* distribution on kelp thalli regardless of phlorotannin content may indicate that variables other than phlorotannins, such as physical factors like water movement, ultimately influence distribution of this grazer. Alternatively, phlorotannins may not act as feeding-deterrent compounds at all as recently proposed (e.g., Amsler and Fairhead, 2006), which would leave the purpose of differentially allocated phlorotannins to different tissue types as of yet unresolved.

Conditions in the study environment should be conducive to inducible as opposed to constitutive phlorotannin production since the main kelp grazer in the area, *L. vincta*, is only seasonally abundant, is relatively immobile as compared to other herbivores, and its grazing provides a considerable risk to kelp thalli. Induction of phlorotannins in *A. clathratum* and *S. latissima*, however, could not be shown in this study. Alternatively, constitutive phlorotannin production might be predicted if the dominant grazer was large, highly mobile or present in kelp beds throughout the year, such as many fish species (Hay, 1996). During the study period at Hesketh Island and Jakolof Bay, *A. clathratum* and *S. latissima* demonstrated highest phlorotannin contents among the studied kelp species and overall, fewer *L. vincta* were observed on these understory species as compared to the kelp canopy. In addition, *A. clathratum* and *S. latissima* were generally less grazed than other similarly abundant macroalgae in the study environment (Dubois, personal observation). If grazer density is low on *A. clathratum* and *S. latissima* thalli, perhaps due to constitutively high phlorotannin content, then the use of additional inducible phlorotannin production may not be beneficial and may be unnecessarily costly.

The absence of induction in *A. clathratum* and *S. latissima* may also indicate that experimental procedures constrained phlorotannin inducibility. For example, the duration

of the experiment may have been too short and therefore did not allow expression of induction, though this is not likely given the rapid induction previously observed in some kelp tissues (Hammerstrom et al., 1998; Lüder and Clayton, 2004). On the other hand, the sampling interval may have been too large to identify a rapid response that occurred in less than 24 hours, as previously noted by Hammerstrom et al. (1998). Additionally, environmental factors varying between seasons, such as irradiance and nutrient concentrations (reviewed by Amsler and Fairhead, 2006), could have constrained induction. It is also important to note that the considerable variability of phlorotannin content within localized tissue areas may obscure trends otherwise noticeable if variability in localized tissue areas exceeds that of an induced response.

Specificity of the mechanism of wounding required for induction may be high since inducible defenses are presumed most beneficial when elicited by the damage of the predominant grazer in the community (Pavia et al., 1997; Pavia and Toth, 2000; Borell et al., 2004). Different types of grazing damage can be caused by rasping tissue surfaces, biting off parts of a thallus or scraping holes through tissue. *Lacuna vincta* generally scrapes small holes into kelp thalli (Fralick et al., 1974; Johnson and Mann, 1986; Dubois, personal observation). Though simulated grazing in this study seemed to emulate the type of damage caused by *L. vincta*, herbivory by the actual grazer might have caused a different result. For instance, simulated herbivory on *A. nodosum* did not significantly increase phlorotannin content (Pavia et al., 1997; Pavia and Toth, 2000), but grazing by the gastropod *Littorina obtusata* did (Pavia and Brock, 2000; Pavia and Toth, 2000; Toth et al., 2005). However, induction was not observed in *Laminaria hyperborea* laminae when exposed to either *L. vincta* or simulated grazing (Toth and Pavia, 2002a).

In conclusion, the consistent presence of higher phlorotannin content in attachment and meristematic relative to non-meristematic tissues in kelp species from this study seems to provide an effective defense strategy that may improve thallus fitness based on protection of tissues vital to survival and propagation, regardless of *L. vincta* distribution. Additionally, slower-growing kelps may invest more resources into constitutively higher phlorotannin content as compared to rapidly-growing species such

as *N. luetkeana* (see Chapter 2). If small mesograzers like *L. vincta* are effectively deterred by phlorotannin content typical of the more valuable tissues as determined by the ODT for *A. clathratum*, *S. latissima* and *S. subsimplex*, then the use of phlorotannin induction may not be widespread in the Northeastern Pacific. Here, the shallow subtidal is often dominated by a dense understory of these relatively long-lived kelp species and small mesograzers.

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Table 3.1 Repeated-measures ANOVA results of within-subject effects of tissue type on $\sin^{-1}(\sqrt{\ })$-transformed phlorotannin content (% dry mass (DM)) in, and *Lacuna vincta* density (snails 100 cm^{-2}) on, *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* at Hesketh Island and Jakolof Bay.

	Phlorotannin content (% DM)			*L. vincta* density (snails 100 cm^{-2})		
Source	*df*	*F*	*p*	*df*	*F*	*p*
Hesketh Island						
N. luetkeana	4	29.13	< 0.001	3	1.80	0.201
Error	136			42		
A. clathratum	4	80.81	< 0.001	3	6.07	0.003
Error	148			75		
S. latissima	4	176.6	< 0.001	3	3.04	0.051
Error	124			72		
S. subsimplex	4	34.31	< 0.001	3	3.81	0.028
Error	124			60		
Jakolof Bay						
N. luetkeana	4	42.85	< 0.001	3	1.39	0.315
Error	80			33		
A. clathratum	4	109.3	< 0.001	3	6.85	0.004
Error	92			51		
S. latissima	4	48.32	< 0.001	3	4.93	0.014
Error	76			51		
S. subsimplex	4	28.99	< 0.001	3	6.75	0.004
Error	84			51		

Analyses for phlorotannins and snail density were performed separately and for each site and kelp species. Tissue types include holdfast (phlorotannin analyses only), meristematic stipe (phlorotannin analyses only) or entire stipe (*L. vincta* analyses only), meristematic blade, non-meristematic blade and degenerating blade.

Table 3.2 Repeated-measures ANOVA results of within-subject effects of tissue type on the ratio of *Lacuna vincta* density to phlorotannin content in *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* at Hesketh Island and Jakolof Bay.

	L. vincta density : phlorotannin content		
Source	*df*	*F*	*p*
Hesketh Island			
N. luetkeana	3	2.43	0.120
Error	39		
A. clathratum	3	5.73	0.004
Error	78		
S. latissima	3	3.04	0.052
Error	69		
S. subsimplex	3	5.12	0.011
Error	54		
Jakolof Bay			
N. luetkeana	3	2.66	0.160
Error	21		
A. clathratum	3	5.31	0.012
Error	48		
S. latissima	3	5.89	0.009
Error	45		
S. subsimplex	3	5.94	0.009
Error	45		

Analyses were performed separately for each site and kelp species. Tissue types include holdfast, stipe, meristematic blade, non-meristematic blade and degenerating blade.

Table 3.3 Pearson's Product Moment correlation results of *Lacuna vincta* density vs. phlorotannin content in *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* at Hesketh Island and Jakolof Bay.

	L. vincta density (snails 100 cm^{-2}) vs. phlorotannin content (% DM)		
Site, kelp species	*n*	*r*	*p*
Hesketh Island			
N. luetkeana	60	0.079	0.546
A. clathratum	105	-0.280	0.004
S. latissima	95	-0.549	<0.001
S. subsimplex	76	0.121	0.298
Jakolof Bay			
N. luetkeana	41	0.342	0.029
A. clathratum	72	-0.183	0.123
S. latissima	63	-0.530	<0.001
S. subsimplex	68	0.240	0.049

Analyses were performed separately for each site and kelp species. Data are grouped for all tissue types.

Table 3.4 Repeated-measures ANOVA results of within-subject effects of time (day 0-7) on phlorotannin content based on mechanical wounding of *Agarum clathratum* and *Saccharina latissima* at Hesketh Island and Jakolof Bay.

	Phlorotannin content (% DM)		
Source	*df*	*F*	*p*
Hesketh Island			
A. clathratum	7	1.07	0.401
Error	35		
S. latissima	7	1.79	0.142
Error	21		
Jakolof Bay			
A. clathratum	7	2.46	0.027
Error	63		
S. latissima	7	2.54	0.028
Error	42		

Analyses were performed separately for each kelp species and site.

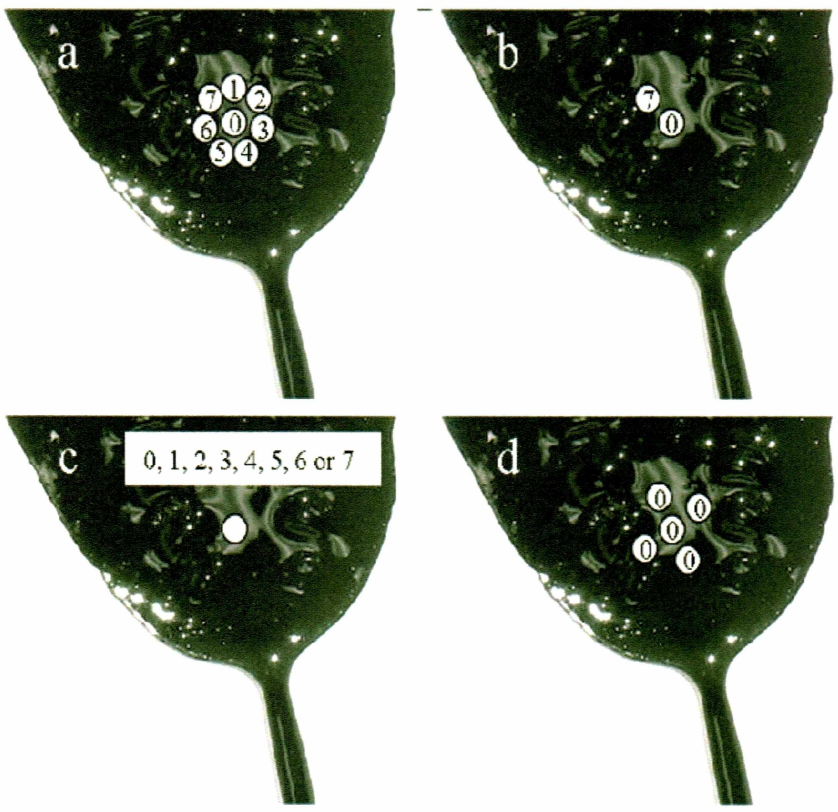

Fig. 3.1 Induction study design. Treatment thalli were wounded a) repeatedly from day 0 through day 7 or b) once at day 0 and again at day 7. Control thalli were wounded c) once each at day 0, 1, 2, etc. to establish baseline phlorotannin content or d) five times within a localized tissue area at day 0. Numbers (0-7) indicate day of sampling in terms of 24 hour increments, with day 0 as the beginning of the experiment and day 7 as the end.

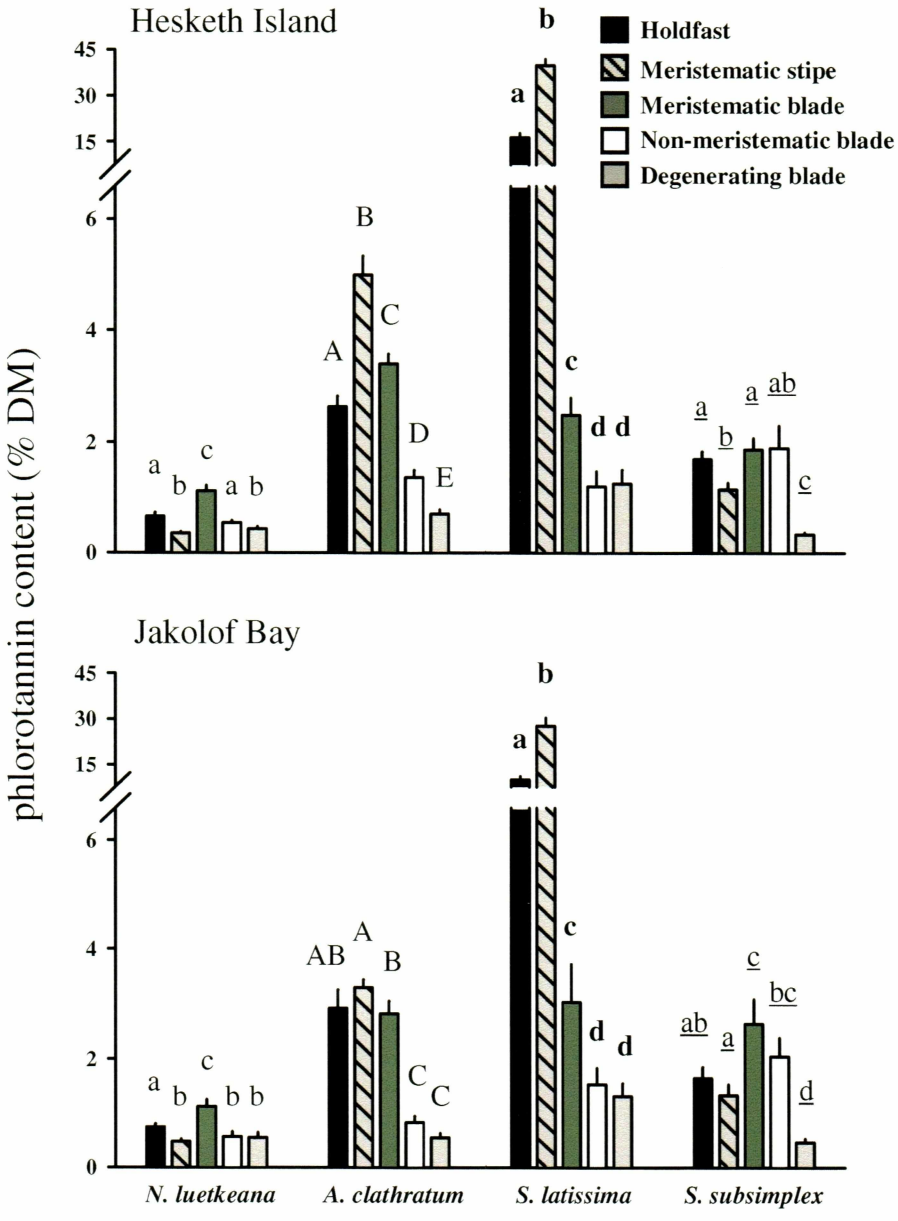

Fig. 3.2 Phlorotannin content (% dry mass (DM); mean ± s.e.) in *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* thalli at Hesketh Island (n = 34-39 per tissue type) and Jakolof Bay (n = 20-24 per tissue type). Phlorotannin content is shown for five tissue types (holdfast, meristematic stipe, meristematic blade, non-meristematic blade, degenerating blade). Due to low sample size and inconsistent presence between kelp species, phlorotannin content in reproductive tissue is not shown. Different letters indicate significant differences (repeated-measures ANOVA, $p < 0.05$) between tissue types within each kelp species at each site. Note breaks in y-axes.

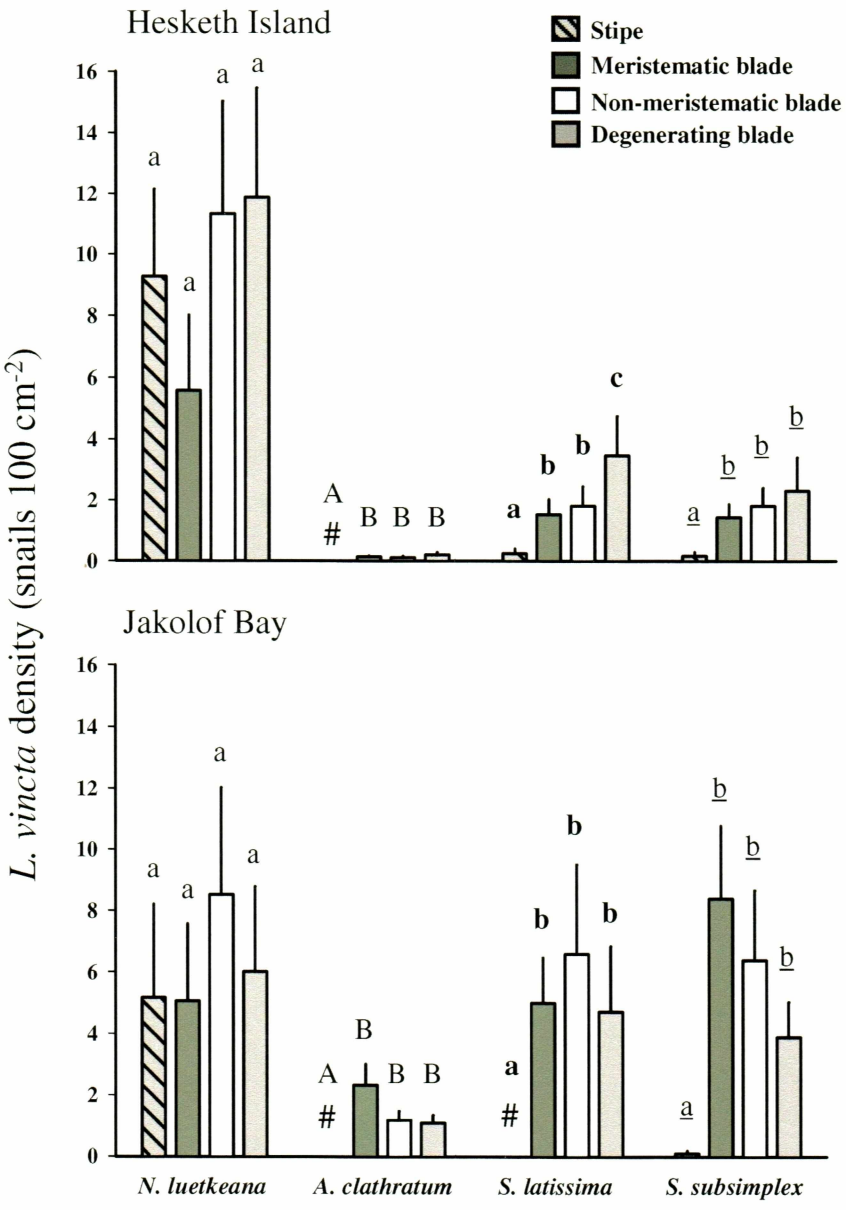

Fig. 3.3 *Lacuna vincta* density (snails 100 cm^{-2}; mean ± s.e.) on *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* thalli at Hesketh Island (n = 15-27 per tissue type) and Jakolof Bay (n = 12-18 per tissue type). Snail density is shown on four tissue types (stipe, meristematic blade, non-meristematic blade, degenerating blade). Holdfast tissue is not shown since no *L. vincta* were observed on this tissue type on any sampling occasion. # represents the absence of snails on stipe tissue. Different letters indicate significant differences (repeated-measures ANOVA, $p < 0.05$) between tissue types within each kelp species at each site.

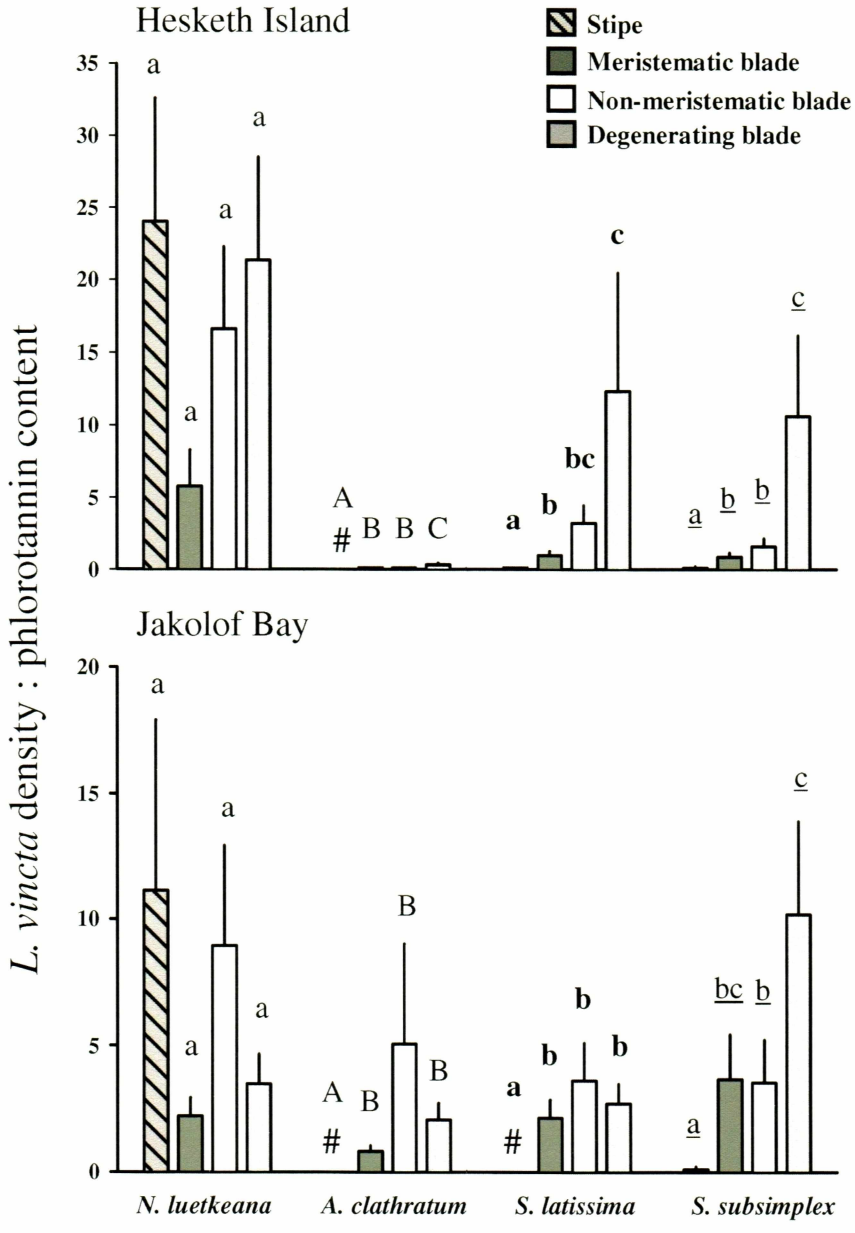

Fig. 3.4 Ratio of *Lacuna vincta* density to phlorotannin content (mean ± s.e.) on *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* thalli at Hesketh Island (n = 15-26 per tissue type) and Jakolof Bay (n = 9-18 per tissue type). Plots show ratios for stipe, meristematic blade, non-meristematic blade and degenerating blade tissue for each kelp species and site. # represents the absence of snails on stipe tissue and hence ratio values. Different letters indicate significant differences (repeated-measures ANOVA, $p < 0.05$) between tissue types within each kelp species at each site. Note difference in y-axes.

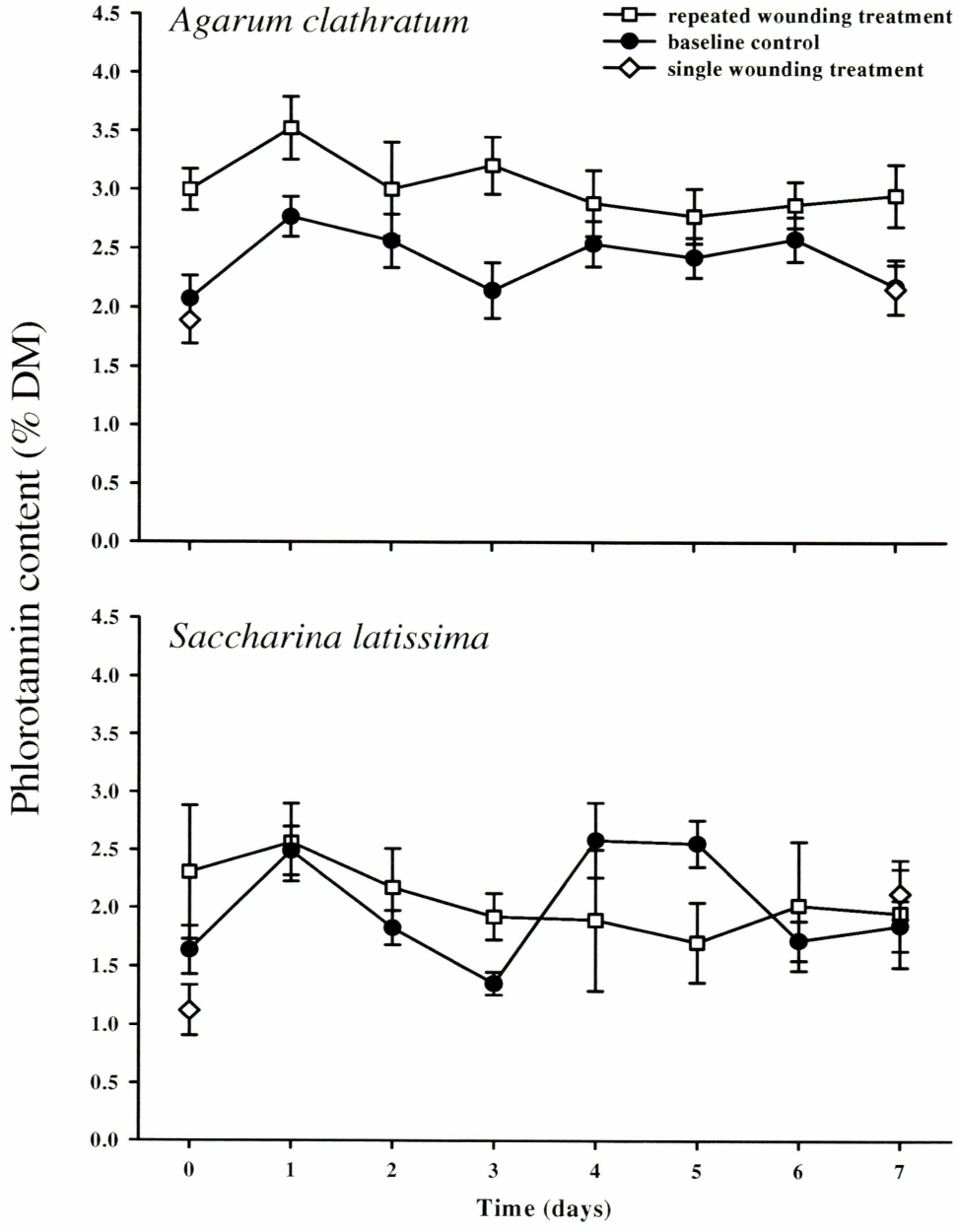

Fig. 3.5 Phlorotannin content (% dry mass (DM); mean ± s.e.) in *Agarum clathratum* (n = 10 per day) and *Saccharina latissima* (n = 8 per day) from week-long induction study at Hesketh Island. Repeated wounding treatment corresponds to the same thalli punched repeatedly from day 0 through day 7. Baseline control represents previously undamaged thalli punched once to establish background phlorotannin content throughout the experiment. Single wounding treatment indicates phlorotannin content in thalli punched on day 0 and 7.

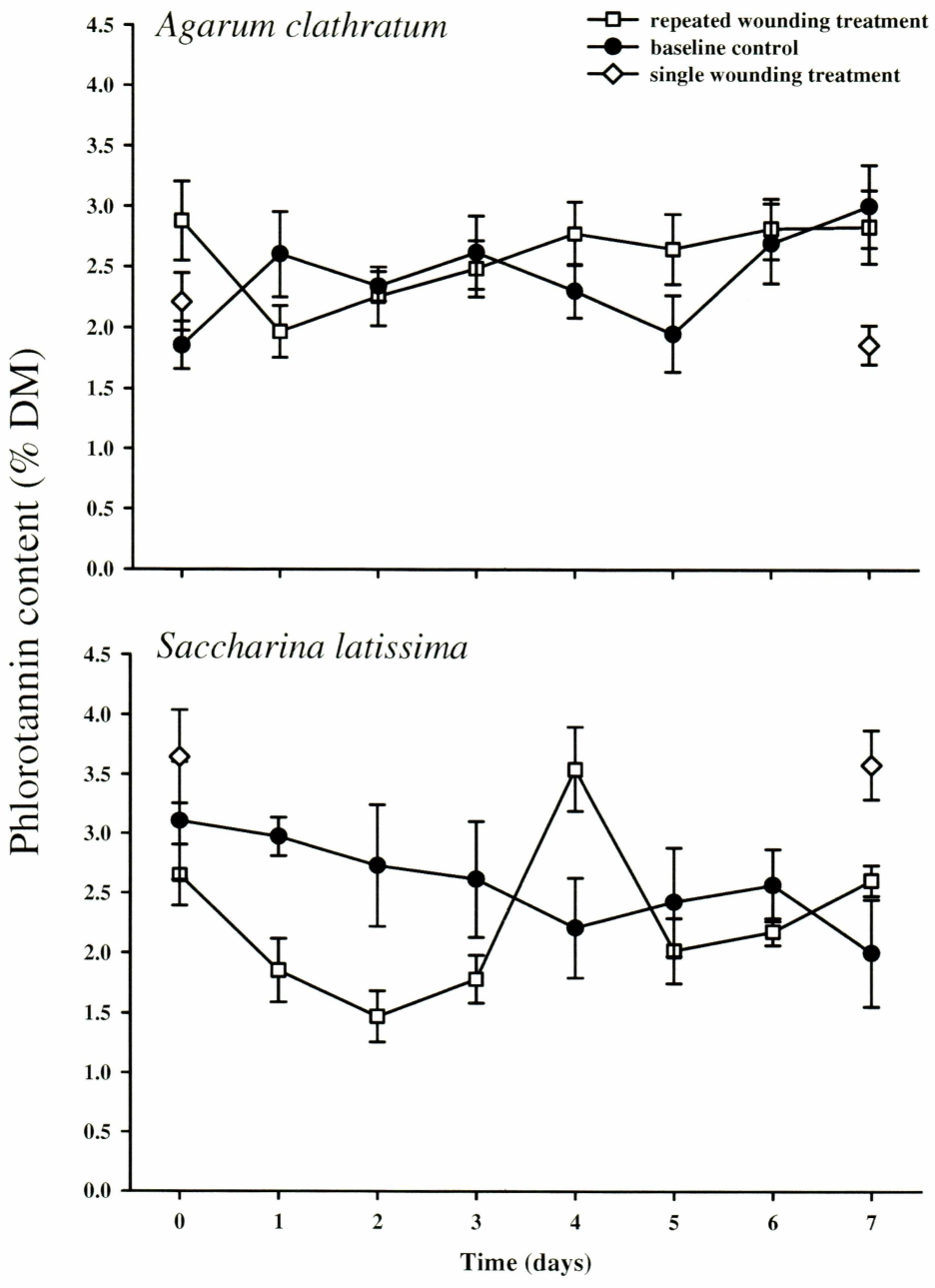

Fig. 3.6 Phlorotannin content (% dry mass (DM); mean ± s.e.) in *Agarum clathratum* (n = 10 per day) and *Saccharina latissima* (n = 8 per day) from week-long induction study at Jakolof Bay. Repeated wounding treatment corresponds to the same thalli punched repeatedly from day 0 through day 7. Baseline control represents previously undamaged thalli punched once to establish background phlorotannin content throughout the experiment. Single wounding treatment indicates phlorotannin content in thalli punched on day 0 and 7.

Chapter 4 - General conclusions

The overarching study hypothesis was that an inverse relationship exists between kelp phlorotannin distribution and the habitat and food preferences of the grazer *Lacuna vincta*. This study has shown that patterns of phlorotannin content and distribution vary on multiple scales. On a temporal scale, phlorotannins are greatly influenced by life history strategy, as evidenced by distinct differences in stipe and blade tissue phlorotannin content between the annual canopy-forming species *Nereocystis luetkeana* and perennial understory kelps *Agarum clathratum*, *Saccharina latissima* and *S. subsimplex* when exposed to the same grazer and environmental regimes. However, differences in life history do not seem to affect the spatial within-thallus distribution of phlorotannins since each studied kelp species similarly demonstrated high proportional phlorotannin allocation to attachment and meristematic tissues. Spatial allocation of phlorotannins was instead associated with the presumed relative importance of differentiated tissue types to the fitness of individual thalli as outlined by the Optimal Defense Theory.

Density and distribution of the gastropod grazer *Lacuna vincta* in the study environment were not strongly associated with phlorotannin distribution among tissue types within kelp thalli, though preferences of *L. vincta* for particular kelp species were generally reflected in feeding assays. Correlative inferences from field observations and palatability assays suggest that *L. vincta* is not significantly deterred by phlorotannin content of less than ~2 % dry mass, though the deterrent effects of phlorotannins were not specifically tested. Nevertheless, the presence of kelp phlorotannins even at low concentrations presumably decreases mass of tissue lost to grazing by *L. vincta* to some extent. The factors influencing distribution of this dominant grazer on kelp thalli are likely numerous and may also include nutritive value and toughness of tissue. However, all variables involved in determining *L. vincta* distribution are likely superseded by physical stresses such as currents and wave exposure.

The relationship between kelp thalli phlorotannins and grazers is highly complex and can vary between kelp species, physical location and season. The influence of abundant grazers such as *L. vincta* on the fitness of individual thalli may ultimately contribute to the variability of kelp bed dynamics and possibly alter the composition of the subtidal community. To estimate the order of magnitude of this grazer's influence, rough approximations were made based on feeding rates of *L. vincta* on each kelp species and the average density of snails and kelp thalli during the summer of 2005. These estimates show that *L. vincta* could have feasibly grazed about 45 % of the total *N. luetkeana* tissue m^{-2} in an average summer month at Hesketh Island. The relative impact of *L. vincta* grazing on *N. luetkeana* thalli was likely much lower at Jakolof Bay (only about 2 % tissue m^{-2} $month^{-1}$) due to the decreased density of *L. vincta* in the canopy and the greater *N. luetkeana* density at this site. Such differences in grazer impact could explain variability of kelp canopies on regional scales. Grazing effects on the understory kelp species with higher phlorotannin content were generally much lower, ranging from 0.25 % to 3.5 % of total tissue consumed m^{-2} $month^{-1}$, with slightly greater grazing impacts at Jakolof Bay. Given these extrapolations, the destructive capabilities of *L. vincta* on kelp communities, especially the low-phlorotannin kelp canopy, seem undisputable and are well illustrated by the potential tissue loss due to grazing by this herbivore.

Any amount of feeding deterrence caused by the presence of phlorotannins could be beneficial to the maintenance of kelp tissues. Knowledge of the patterns of phlorotannin content and distribution in dominant kelp species and the effects they have on grazer distribution and feeding are vital to a true understanding of the factors influencing kelp density and composition. However, the loose association shown in this study between phlorotannin content and grazer habitat and food preferences challenges the traditional consideration of these compounds as feeding deterrents and brings into question the role of phlorotannins within kelp tissues. It is possible that first and foremost phlorotannins serve other primary or secondary roles such as cell wall strengthening and antifouling/UV protection, respectively, while inadvertently deterring feeding by grazers

at higher concentrations. Based on the multi-scale variability in phlorotannin patterns observed in this study, a comprehensive assessment of the role of phlorotannins in kelp, therefore, would require the use of numerous species in distinct geographic regions that are exposed to a wide range of physical and biotic factors. Furthermore, the coupling of traditional ecological approaches with molecular techniques assessing carbon assimilation and phlorotannin turnover rates would provide added insight as to phlorotannin function within thalli and thus, how these compounds ultimately benefit individual fitness.